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Pork Adrenal-Cortex Extract: Effect upon Carbohydrate Metabolism and Work Capacity in Addison's Disease[1]

CYRIL M. MACBRYDE, M.D., AND
F. A. DE LA BALZE, M.D.

From the Department of Internal Medicine, Washington University School of Medicine, the Barnes Hospital, and the Washington University Clinics, St. Louis, Missouri

EXTENSIVE experimental and clinical evidence indicates that the adrenal cortex plays a direct and positive role in the control of carbohydrate metabolism, but the mechanism of the control is not clear and many of the collected data are contradictory. No attempt will be made to review all of the pertinent literature, since this has been well done elsewhere (1). The purpose of this report is to emphasize the inadequacy of the present methods of treating Addison's disease, especially in regard to the deranged carbohydrate metabolism, and to describe studies with pork adrenal-cortex extract. This extract has for the first time given us a means of restoring the carbohydrate metabolism to normal.

Among 28 patients with Addison's disease whom we have studied, the fasting blood-sugar level of 13 has been lower than average normal. In seven of these patients hypoglycemia has been severe enough to cause frequent symptoms and definite muscular weakness. These symptoms have continued to occur in spite of adequate control of the electrolyte and water metabolism and of the blood pressure with desoxycorticosterone or beef adrenal-cortex extract. In an effort to find a satisfactory method of maintaining normal glycemia, comparative studies of beef and pork adrenal-cortex extracts were performed upon these seven patients with Addison's disease.

The principal alterations in carbohydrate

[1] A preliminary report of these studies was presented at the Sixteenth Annual Meeting of the Central Society for Clinical Research, Chicago, November 5, 1943.

metabolism which have been described in patients with Addison's disease are: (1) low fasting morning blood sugar; (2) flat oral glucose tolerance curve; (3) hypoglycemia following oral or intravenous administration of glucose; (4) severe hypoglycemia during fever or infections or after prolonged fasting. In adrenalectomized animals hypoglycemia occurs and there is depletion of the tissue carbohydrate stores. In such animals the blood sugar and the liver and muscle glycogen can be restored to normal or raised to supranormal levels with adrenal-cortex extract. Desoxycorticosterone, which is highly effective in correcting the abnormalities in salt and water metabolism, is conspicuously lacking in influence on the carbohydrate derangement. There has been no adequate clinical treatment for relief of patients suffering with hypoglycemic symptoms. Avoidance of severe muscular exertion is helpful. Elimination of large meals and pure sugars and the use of small, frequent meals, high in protein and containing slowly absorbable carbohydrate, are valuable measures. In our experience beef adrenal-cortex extract has a relatively slight and transient effect upon the blood sugar, even in the large doses used in treating crisis.

The biological activity of beef adrenal-cortex extract as compared to that made from the adrenal glands of hogs was studied by Kuizenga, Wick, Ingle, Nelson and Cartland (2). The rat-survival test and the Ingle muscle-contraction test were used as measures of activity and by both tests the pork extract revealed a higher potency. The most marked differences were apparent in the muscle-contraction test, indicating that the greater effect of the pork extract probably results from its higher content of those adrenal steroids which bear an oxygen atom at carbon atom 11 and are active in carbohydrate metabolism.[2] This report led us to undertake studies of the effect of pork adrenal-cortex extract upon the blood sugar and upon muscular efficiency in Addison's disease. No previous reports of the clinical use of pork adrenal-cortex extract have appeared except our own preliminary communication (3).

MATERIALS AND METHODS

Seven patients with Addison's disease were studied. Four were men and two were women and their ages ranged from 20 to 42 years. One was a seven-month-old, female child. All had typical signs and symptoms and all usually exhibited lower-than-normal, fasting blood sugar. One patient (J. O.) suffered from typical hypoglycemic attacks with loss of consciousness. The diets employed were those considered optimum for each patient and were not altered throughout the study. Sodium, potassium and chloride intake were carefully measured and were not changed. Water and electrolyte balance were restored as nearly as possible to normal with sodium chloride and desoxycorticosterone therapy before comparative study of the effects of the extracts was undertaken.

The beef adrenal-cortex extract[3] employed has proved highly effective in the treatment of the crises of Addison's disease when 20 cc. to 120 cc. is given in the first 24 hours. All seven of the patients included in this study have at some time recovered from crisis after treatment with this extract. For maintenance therapy, intramuscular injection of 2 to 5 cc. daily has been sufficient in several of these patients to establish normal blood pressure and normal salt and water balance. Each cc. of the beef extract is derived from 75 gm. of fresh gland. Although no definite statement is made concerning the potency of this extract in units, a safe estimate is that it contains not less than 4 rat units per cc.

The pork adrenal-cortex extract[4] contains 40 rat units per cc. By physiological assay each cc. is equivalent to approximately 2 mg. of 17-oxy-11-dehydrocorticosterone (compound E) or 4 mg. of corticosterone. The more concentrated pork extract is put in oil, whereas the beef extract is aqueous. It is important to note that the relative number of effective units in each cc. of the two extracts does not denote

[2] Dr. M. H. Kuizenga reported at the Gibson Island Hormone Conference, 1943, that much larger amounts of the C_{11}-oxygenated steroids can be isolated from pork glands (unpublished data).

[3] We are indebted to Dr. David Klein of the Wilson Laboratories, Chicago, for generous supplies of Wilson's Adrenal-Cortex Extract used in these studies.

[4] The pork adrenal-cortex extract was supplied through the courtesy of Dr. E. Gifford Upjohn of the Upjohn Company, Kalamazoo, Michigan.

the relative potencies of beef and pork cortical extracts but indicates only that the pork extract was prepared in a more concentrated form. One cc. of the pork extract (in oil) represents approximately the same survival-growth potency (40 rat units) as 10 cc. of beef extract (aqueous). In the following experiments the extracts were as a rule given in these proportions (usually 2 cc. and 20 cc. respectively) intramuscularly, so that the electrolyte factors would be given in the same dose. Any significant difference in the blood-sugar levels obtained with the two extracts may, therefore, be logically interpreted as due to a difference in the content of carbohydrate factors.[5]

PLAN OF EXPERIMENTS

(1) *Fasting Blood Sugar and Blood Sugar Throughout the 24 Hours.* The blood sugar before breakfast was determined with the usual diet when the treatment was salt and desoxycorticosterone alone. The blood glucose content was then measured before breakfast following days on which the patient had been treated with various doses of beef or of pork extract.

The venous blood-sugar curve was followed throughout the 24 hours when the patient was taking the usual meals at 8 A.M., 12 noon, and 5:30 P.M. Blood samples were obtained at 7:30 A.M. (fasting), 10:30 A.M., 2:30 P.M., 4:30 P.M., 7:30 P.M., 12 midnight and again at 7:30 A.M. (fasting). The 24-hour blood-sugar curve was determined upon the same patient under various conditions: (a) when treated with desoxycorticosterone and salt alone; (b) when 1 cc. of pork extract was given intramuscularly at 6:30 A.M.; (c) when 10 cc. of beef extract was given intramuscularly at 6:30 A.M.; (d) when 4 cc. of pork extract was given at 6:30 A.M.; (e) when 12 cc. of pork extract was given in divided doses of 4 cc. each at 6:30 A.M.; 1:30 P.M., and 6:30 P.M.

(2) *Glucose-Tolerance Curves.* (*a*) *Oral:* The patients were given glucose in 40 per cent solution with lemon juice, allowing 1 gm. of glucose per kg. of body weight. The curve of the venous blood sugar was determined fasting, and one-half hour, one, two, and three hours after the glucose administration. Curves were obtained without added treatment, with 1 cc. of pork extract, with 4 cc. of pork extract, or with 20 cc. of beef extract given intramuscularly at 6:30 A.M. (*b*) *Intravenous:* A fasting blood sample was taken at 8:30 A.M. and another at 9:30 A.M. Immediately thereafter 0.5 gm. of glucose per kg. body weight was given in 20 per cent solution in distilled water during a period of 30 minutes. Blood samples were taken at one-hour intervals for the next three hours. For comparison with this control test, curves were determined on the same patient when 1 cc. of pork extract or 10 cc. of beef extract was given intramuscularly immediately after the first fasting blood-sugar sample was obtained.

(3) *Arterio-Venous Differences in the Blood Sugar.* It was unexpectedly found that the venous blood sugar in fasting patients usually showed no change after beef extract but fell after pork extract. We, therefore, obtained capillary blood-sugar specimens at the same time venous blood was secured to determine the changes in the arterial blood and in the arterio-venous difference which might follow injection of the adrenal-cortex extracts. This was done during fasting and after food intake. Samples of capillary and venous blood were obtained at 6:30 A.M. and the patient was given either 2 cc. of pork extract or 20 cc. of beef extract. One hour later samples were again obtained and the patient was given breakfast. Two and one-half hours after breakfast (at 10:30 A.M.) capillary and venous blood samples were again secured.

In another series of tests, the patients were kept fasting throughout and capillary and venous blood sugar were determined one hour and three hours after 2 cc. or 7 cc. of pork extract, or 20 cc. of beef extract.

(4) *Work Capacity Tests.* The ability to perform muscular work was tested in two ways, one involving the flexors of the forefinger of the right hand only and the other involving all the muscle groups in both legs.

(a) An ergometer was so arranged that flexion of the forefinger lifted a 2.065 kg. weight. Flexion was performed at the rate of one every two seconds (30 per minute). The number of

[5] Dr. Dwight Ingle (unpublished data) found that beef extract in oil and aqueous beef extract show no differences in assay value in his work test. Therefore the differences in media cannot account for the greater effect of the pork extract.

contractions performed, the time for which the exercise could be continued and the excursion of the weight were carefully measured. From these figures it was possible to compute the amount of work performed and to determine

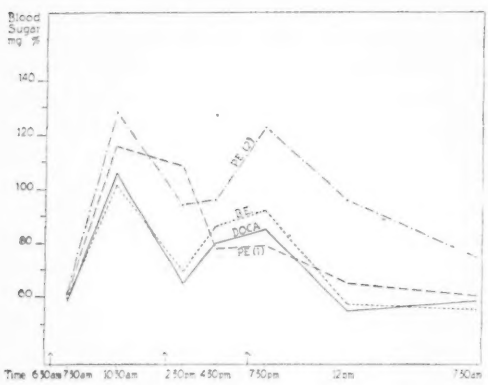

FIG. 1. Comparative 24-hour blood-sugar curves with D O C A, with beef extract (10 cc. intramuscularly at 6:30 A.M.) and with pork extract. (P.E. [1] after 1 cc. intramuscularly at 6:30 A.M.; P.E. [2] after 4 cc. at 6:30 A.M., 1:30 P.M., and 6:30 P.M.).

the effect of the various types of treatment upon work performance.

(b) Ability to climb stairs at a rapid, fixed rate was measured before and after the various types of treatment. By multiplying the patient's weight by the height of the stairs climbed the comparative amounts of work in kilogram-meters were obtained.

(5) *Blood and Urine Chemistry.* The concentration in the serum of potassium, sodium and chloride was determined frequently in all patients, at times appropriate to judge the effect of therapy. Urinary-nitrogen excretion and chloride excretion were measured in two cases during the experimental period.

RESULTS

(1) *Fasting Blood Sugar and 24-Hour Blood Sugar Curves.* When patients were maintained with D O C A pellets and salt alone, the blood-sugar curves showed low levels occurring several hours after the last meal, this tendency being most apparent in the midnight and fasting blood-sugar levels (fig. 1). When 10 cc. of beef extract was given, the curves differed little from those obtained with D O C A alone. With a single injection of 1 cc. of pork adrenal-cortex extract, however, the rise after food intake was greater and for approximately ten hours after the injection the tendency to elevated blood sugar was evident. By midnight, 17½ hours after the injection, the blood sugar had not returned quite to the low levels observed with D O C A or beef extract. When the pork extract was given in larger doses, such as 4 cc., (fig. 1), the rise after food intake was greater than after 1 cc., reaching levels higher than normal and remaining high for some hours. Low midnight and fasting blood-sugar levels did not occur when 4 cc. was given intramuscularly at 6:30 P.M., the last food being taken at 5:30 P.M. When 4 cc. of the pork extract was given only once, at 6:30 A.M., most of the evidence of its effect on the blood sugar was gone within about 10 hours. The maximum effect occurred about two to four hours after the injection either of 1 cc. or of 4 cc. When 4 cc. of the pork extract was given three times during the day, a moderate hyperglycemia was present throughout the day but the midnight blood glucose and the fasting blood sugar the next morning were within normal limits.

(2) *Glucose-Tolerance Curves.* (*a*) *Oral.* There was little difference in the glucose-tolerance curves when patients were given 20 cc. of beef extract intramuscularly from those obtained during pellet and salt treatment alone (fig. 2), except for a tendency to prevention of the usual hypoglycemia occurring at the second hour. After 1 cc. of pork extract there was a

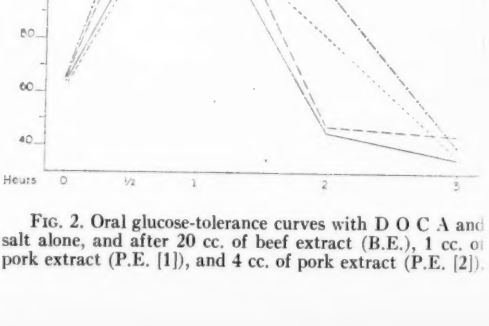

FIG. 2. Oral glucose-tolerance curves with D O C A and salt alone, and after 20 cc. of beef extract (B.E.), 1 cc. of pork extract (P.E. [1]), and 4 cc. of pork extract (P.E. [2]).

greater rise, but a rapid fall in the blood sugar was evident at the second hour. When 4 cc. of the pork extract was given, the blood sugar increase was still greater and the fall was postponed, the two-hour blood sugar being considerably higher than the fasting level and averaging 20 mg. per cent higher than after the beef extract. Hypoglycemia was evident in every instance at the third hour, the degree not significantly differing from that appearing with salt and D O C A therapy alone. (*b*) *Intravenous.* The intravenous glucose tolerance tests showed no significant increase following the injection of either pork or beef cortical extract at

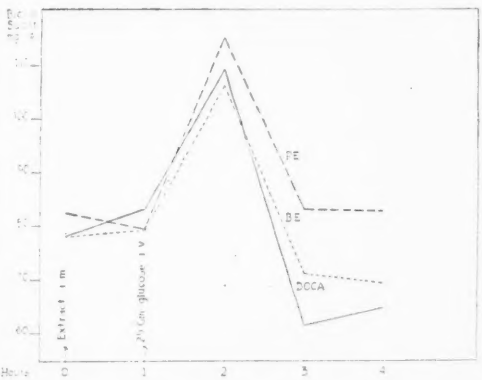

FIG. 3. Intravenous glucose-tolerance tests with D O C A, with 10 cc. of beef extract (B.E.) and with 1 cc. of pork extract (P.E.).

the first hour but at the second and third hours there was a definite prevention of the hypoglycemic phase with the pork extract. The beef extract produced slightly higher blood-sugar levels at the second and third hours after the intravenous glucose than those which occurred with D O C A alone.

(3) *Arterio-Venous Differences in the Blood Sugar.* We had expected to find that the venous blood sugar during fasting would rise after pork-extract injection. This did not occur but a consistent decrease in the blood-glucose content was observed, the fall being greater with increasing doses (fig. 4). With beef extract there was no significant change.

The capillary blood-sugar levels, however, increased definitely after pork extract, while a slight fall or no change was noted after the beef extract (fig. 5). The rise in the capillary

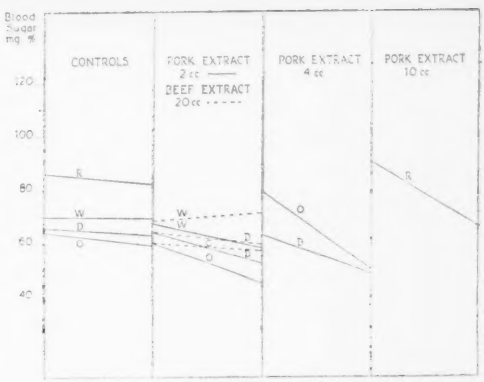

FIG. 4. Fasting venous blood-sugar levels showed no change during one hour after injection of beef cortical extract, but fell after pork extract. The letters W, D, O, and R indicate comparative determinations upon patients with these initials.

blood sugar and the fall in the venous blood sugar resulted in a considerable increase in the arterio-venous difference following pork extract. There was a fall or no change in the A-V difference after beef extract (fig. 6).

(4) *Work-Capacity Tests.* (*a*) *Finger ergometer.* In table 1 the results of injections of pork and beef extract upon work capacity in this test are seen to differ greatly. In five trials upon three patients the beef extract gave no improvement three times; in two instances gains of 10 per cent and 18 per cent were registered. The pork extract, however, increased

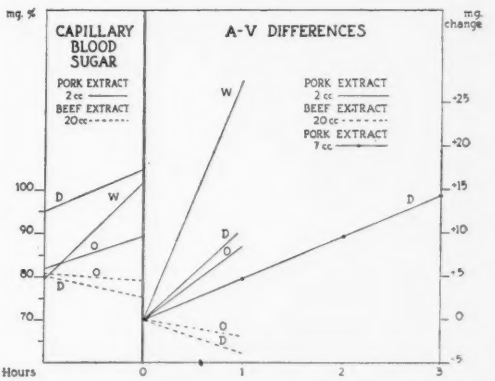

FIG. 5. Capillary blood-sugar values: rise after pork extract; no rise after beef extract.

FIG. 6. Change in arterio-venous differences during one hour and three hours (fasting). Consistent rise after pork extract. Fall or no change after beef extract. The letters W, D, and O indicate comparative determinations on patients with these initials.

TABLE 1. WORK-CAPACITY TESTS: FINGER ERGOMETER

PATIENT	WORK IN KILOGRAM-METERS	PER CENT IMPROVE-MENT
J.O.		
Control (1)	6.27	
(2)	5.98	
2 hours after		
20 cc. B. E. (1)	6.39	0
(2)	7.06	18
2 hours after		
2 cc. P. E. (1)	9.91	58
(2)	11.77	97
J.D.		
Control	31.59	
2 hours after		
20 cc. B. E.	31.09	0
2 hours after		
2 cc. P. E.	45.09	43
W.B.		
Control (1)	10.78	
(2)	4.73	
2 hours after		
20 cc. B. E. (1)	9.65	0
(2)	5.26	10
2 hours after		
2 cc. P. E. (1)	13.63	26
(2)	7.37	62

work capacity in every one of five tests, the greater work performed ranging from 26 per cent to 97 per cent. (*b*) *Stair climbing.* When ability to perform work was tested by stair climbing, no improvement was observed after beef extract but two of three patients showed definite improvement after pork extract (table 2). Intravenous glucose (25 gm. given over a period of 30 minutes immediately preceding the test) improved work capacity by the same degree (24 per cent) as 2 cc. of pork extract given intramuscularly two hours before the experiment.

TABLE 2. WORK-CAPACITY TESTS: STAIR CLIMBING

PATIENT	WORK IN KILOGRAM-METERS	PER CENT IMPROVE-MENT
P.R.		
Control	523.2	
1 hour after pork extract	422.3	0
J.D.		
Control	707.2	
1 hour after pork extract	795.6	13
W.B.		
Control	843.2	
2 hours after 20 cc. B. E.	843.2	0
2 hours after 2 cc. P. E.	1054.0	24
Immediately after 25 gm. glucose intravenously	1054.0	24

(5) *Blood and Urine Chemistry.* Moderate daily doses of pork adrenal-cortex extract (1 or 2 cc. daily) did not produce an increase in the urinary nitrogen. Large doses, however, increased the nitrogen output in the urine. In patient J. O. the average daily control urinary nitrogen was 8.2 gm., whereas during twelve days on 2 cc. pork extract daily the average output was 8.5 gm. Patient J. D. had a control nitrogen excretion of 6.5 gm.; with 2 cc. pork extract daily, nitrogen excretion was 6.3 gm. However, with 12 cc. daily (three doses of 4 cc.) these patients excreted 9.3 gm. and 12.6 gm. respectively. Twenty cc. of beef extract produced no increase in urinary nitrogen.

The urinary chloride excretion was within normal limits in these two patients regulated with desoxycorticosterone and no significant change was noted after beef- or pork-extract injections.

TABLE 3. PORK-EXTRACT EFFECT ON SERUM ELECTROLYTES

PATIENT	CONDITIONS	Na	K	NaCl
A.W.	Before treatment	312	23.8	560
	After 2 cc. P. E.	325	21.5	624
R.B.	Before treatment	306	24.1	562
	After 2 cc. P. E.	330	20.0	634

In five of the seven patients the blood-sodium, blood-potassium and blood-chloride levels remained within normal limits throughout the studies. Two patients became partly "decompensated," so far as electrolyte control was concerned, for a day or two during the control period. After 2 cc. of pork extract intramuscularly there was in both instances a rise in the serum Na and NaCl, and a fall in the serum K (table 3), occurring within 24 hours.

We have since maintained several patients in good condition and with normal serum electrolytes by giving daily injections of 1 or 2 cc. of the pork extract intramuscularly per day. Sodium chloride in doses of 4 to 6 gm. daily, in addition to a total of 5 to 8 gm. in and on the food, has been the only supplementary treatment in three such patients previously requiring from 2 to 4 mg. daily of desoxycorticosterone with the same intake of salt.

DISCUSSION

In these studies the non-specific influence of severely deranged water and electrolyte metabolism upon the absorption of glucose and upon the ability of the tissues to store glycogen was eliminated by establishing good control of these factors before the experiments were performed. In each case approximately normal blood pressure and normal serum electrolytes and water balance were first obtained with D O C A and salt therapy so that subsequently the cortical extracts could be compared directly as the only varying factor in the therapy.

Increased Glucose Absorption. Pork adrenal-cortex extract caused an increased absorption of glucose both after the usual meals (fig. 1) and after oral glucose-tolerance tests (fig. 2). In each instance the blood glucose rise after the ingestion of carbohydrate was somewhat greater after 4 cc. of the extract than after 1 cc. Beef adrenal-cortex extract failed to cause a similar rise when given in comparable doses [1 cc. of pork extract and 10 cc. of beef extract (fig. 1)] or even when given in twice the comparable amount [1 cc. and 20 cc. respectively (fig. 2)]. It would, therefore, seem that pork adrenal-cortex extract greatly improves carbohydrate absorption from the gastro-intestinal tract, while beef extract has comparatively little such effect. The conclusion that the greater postprandial rise of blood glucose after pork extract indicates increased absorption rather than increased mobilization is supported by the observations in the intravenous glucose-tolerance tests. The rises in this test are practically the same after D O C A alone and after 1 cc. of pork extract or 10 cc. of beef extract (fig. 3). Since the increase in blood sugar after intravenous glucose is approximately the same whether preceded by cortical-extract injection or not, the greater rise produced by cortical extract when carbohydrate is ingested must be due to improved absorption.

Prevention of Post-Absorptive Hypoglycemia. The fall to hypoglycemic levels most apparent two or three hours after the intake of glucose is observed in all patients treated with D O C A and salt alone, whether the carbohydrate is taken as food (fig. 1), oral glucose solution (fig. 2) or intravenous glucose solution (fig. 3). Beef adrenal-cortex extract showed a definite, although less marked effect than pork extract in counteracting this fall when used in the comparable doses of 10 cc. and 1 cc. (figs. 1, 3). When used in twice this proportion (fig. 2), the beef extract was very effective in preventing the hypoglycemia at the second hour.

When carbohydrate is taken either orally or intravenously the blood-sugar level rises. As a result of this rise the output of glucose from the liver is decreased and a post-absorptive decrease in blood glucose, therefore, occurs. This phenomenon is apparent even after pancreatectomy, as shown by Soskin (4). The fall in the peripheral venous blood sugar following a rise may also occur because of increased insulin secretion resulting from a rise in the glucose content of the arterial blood reaching the pancreas (5). The only source of blood glucose during fasting is the liver. The prevention of hypoglycemia following carbohydrate intake in these experiments must, therefore, indicate increased mobilization of glucose from the liver. This mobilization was evident with the beef extract but was greater in degree with comparable amounts of the pork extract. Our clinical observations are supported by the experiments of Selye and Dosne (6) who showed that cortical extract cannot prevent the fall in blood sugar occurring in fasting, adrenalectomized animals unless the liver is present. After hepatectomy the extract has no effect in preventing the hypoglycemia. One may conclude, therefore, that cortical extract does not inhibit the utilization of circulating glucose but increases the glucose output of the liver.

The stimulation of the liver to put out greater amounts of glucose is evident in the midnight and fasting blood-sugar levels following pork-extract injections. At these times, from six hours to fourteen hours after the last food was ingested, the blood glucose was slightly higher than in the control studies even with as little as 1 cc. of pork extract intramuscularly at 6:30 the previous morning. When 4 cc. of the extract was given at 6:30 P.M., the blood sugar at midnight was 42 mg. per cent higher, and at 7:30 A.M. 19 mg. per cent higher than in the control (D O C A) period (fig. 1).

Greater Arterio-Venous Blood-Glucose Dif-

ference. Increased mobilization of glucose during fasting results from pork-extract injections, as shown by the considerable rise in the capillary blood-sugar levels (fig. 5). The fall in the fasting venous blood-glucose levels is greater with increasing doses of pork extract (fig. 4) and there is a considerable increase in the arterio-venous differences of the blood glucose. This probably indicates not only increased mobilization of glucose from the liver, but increased utilization of glucose by the tissues, primarily the muscles. The greater utilization of glucose is shown by the fact that in spite of a larger supply of sugar reaching the muscles (capillary blood from finger-tip) less glucose is returning from the muscles (venous blood from arm vein). The lower venous blood sugar and the greater muscle utilization of glucose suggest that insulin secretion has been increased. This is contradictory to the conclusions of some workers (7) who have interpreted the higher blood-sugar values produced in animals by cortex extracts as evidence of decreased carbohydrate oxidation and interference with insulin activity. From our results it seems more logical to conclude that rather than exerting anti-insulin activity, physiologic amounts of the hormone work synergistically with insulin. The mechanism seems to operate as follows: the cortical hormone causes release of glucose from the liver, the higher blood sugar causes greater insulin secretion and the venous blood sugar falls, which results in greater arterio-venous difference in the blood sugar in consequence of greater utilization by the muscles.

Increased Work Capacity. Possibly not the only cause, but certainly one of the chief causes of weakness and fatigue in Addison's disease is the inability normally to absorb, store, mobilize and utilize glucose. The three groups of experiments so far analyzed in this discussion have shown that pork adrenal-cortex extract will increase the absorption of glucose and its storage, as indicated by the prompt fall after the rise in the blood sugar. Mobilization and utilization are also increased; therefore, ability to perform muscular work should be improved. That work capacity is in fact increased is shown in the fourth group of experiments. Beef extract gave little or no improvement in work performance, just as it gave little or no effect in increasing the absorption or mobilization of glucose. It, therefore, seems likely that the increased capacity to perform muscular work following pork adrenal-cortex extract results from the increased availability of circulating glucose and greater utilization of glucose by the muscles. That availability alone is perhaps the most important factor is indicated by the observation that patient W. B. (table 2) performed as much better after 25 gm. of glucose intravenously as he did after 2 cc. of pork extract intramuscularly. Ingle and and Lukens (8) found that the work capacity of the gastrocnemius muscle of an adrenalectomized rat could be increased to normal by dextrose infusions but not by saline solution. The degree of improvement after glucose was similar to that produced by active cortical steroids.

Source of the Glucose. The source of the increase in available glucose might be either: (1) the glycogen stores of the liver, or (2) accelerated gluconeogenesis in the liver from protein or non-protein precursors. Observations by Long, Katzin and Fry (1) and by Wells and Kendall (7) and others have indicated that when large amounts of cortical extracts are given to experimental animals urinary nitrogen is greatly increased and that the hyperglycemia and glycosuria probably result from accelerated gluconeogenesis from protein. However, Ingle (9) and Kendall (10), in careful quantitative experiments, noted that increased nitrogen excretion from protein destruction could not account for all of the increase in available glucose. Kendall, in fact, showed that under certain conditions little or no increase in nitrogen excretion occurred even after "compound-E" injections. Rats fed a high carbohydrate diet responded to cortical extract by increasing their liver glycogen and muscle glycogen. The extra glucose could not be accounted for by conversion of protein to glucose, since there was comparatively little change in nitrogen excretion (9). The conditions in these animal experiments correspond somewhat to our clinical studies, especially in that the animals were given a liberal carbohydrate intake. The results were similar, also, since in our patients the blood glucose could be elevated above the control levels throughout the twenty-

four hours by injection of 1 or 2 cc. of the pork adrenal-cortex extract daily without increasing the urinary-nitrogen excretion. However, when as much as 12 cc. daily of the pork extract was given (three injections of 4 cc. each) and a moderate hyperglycemia (not sufficient to cause glycosuria) was produced, the nitrogen excretion was considerably increased. It, therefore, seems that the restoration of hypoglycemic to normal levels by pork cortical extract is not accomplished by increased gluconeogenesis from protein, but by better absorption, storage and mobilization of carbohydrate. That the blood sugar may be raised by increased gluconeogenesis from non-protein precursors is suggested by the work of Koepf, Horn, Gemmill and Thorn (11), who found that cortical extract greatly increased the rate of synthesis of carbohydrate from pyruvate and lactate in slices of rat liver. When large doses of extract are employed and hyperglycemia is produced, gluconeogenesis from protein is accelerated and increased protein destruction occurs. In such animal studies, the doses of cortical extracts were far beyond physiological limits and extreme hyperglycemia and glycosuria resulted. In such experiments, especially when made on fasting animals, the greater part of the increased glucose is produced from protein. When unphysiological experiments of this type are performed, producing a diabetes-like state, it is obvious that carbohydrate oxidation will be impaired by such large amounts of cortical extract. Our studies suggest, however, that doses within physiological limits such as would be desirable in therapy of Addison's disease increase the utilization of carbohydrate.

Animal experiments either with intact or adrenalectomized, fasting animals given cortical extracts introduce another dissimilarity from the clinical studies. The only source of carbohydrate in such an animal is body protein, while the regularly fed patient gets glucose from the intestinal tract after meals and can mobilize it from the liver readily, since the liver glycogen stores are frequently replenished.

The low metabolic rate, profound weakness and easy fatigue of Addison's disease certainly do not suggest that the hypoglycemia observed clinically could be the result of excessive carbohydrate utilization by the tissues. The absence of the normal arterio-venous differences in the blood sugar before cortical extract and the restoration of normal or greater than normal A-V differences after pork cortical extract indicates that in adrenal insufficiency there is diminished utilization of glucose by the tissues, which can be corrected by an extract containing enough of the carbohydrate factors.

SUMMARY AND CONCLUSIONS

These studies reveal that pork adrenal-cortex extract has a direct action upon carbohydrate metabolism and that this action is greater than that of beef adrenal-cortex extract. One cc. of the pork extract (in oil) represents approximately the same survival-growth potency (40 rat units) as 10 cc. of beef extract (aqueous). When given intramuscularly in these proportions, usually in doses of 2 cc. and 20 cc. respectively, the pork extract tends to correct the blood-sugar abnormalities of Addison's disease, while beef extract exerts comparatively little such effect.

The pork adrenal-cortex extract produced the following results in studies upon seven patients with Addison's disease: (1) Increased absorption of glucose from the gastro-intestinal tract. (2) Prevention of hypoglycemia after food or glucose intake or as the result of prolonged fasting. This apparently is accomplished by increasing the glucose output from the liver. (3) Increased utilization of glucose by the tissues, as indicated by higher capillary and lower venous blood sugar and greater arterio-venous differences. (4) Increased muscular work capacity. (5) Adequate control of salt and water metabolism, as well as of carbohydrate metabolism, when given in small doses daily.

The greater proportional effect of pork adrenal-cortex extract is apparently due to its higher content of the steroids having an O or OH on C_{11}, such as corticosterone and 17-hydroxy-11-dehydrocorticosterone (Kendall's compound-E).

The extract seems not only to mobilize glycogen from the liver but also to increase the utilization of glucose by the muscles. This is contradictory to the conclusions of some work-

ers who have interpreted the higher blood sugars produced in animals by cortex extracts as evidence of decreased carbohydrate oxidation. Our results favor these conclusions: rather than exerting anti-insulin activity physiologic amounts of the cortical carbohydrate hormone (or hormones) work synergistically with insulin to promote carbohydrate utilization; the cortical hormone causes release of glucose from the liver; the higher blood sugar then causes increased insulin secretion, which results in greater arterio-venous difference in the blood sugar, in consequence of greater utilization by the muscles.

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Growth and Treatment of Dwarfs and Giants[1]

LEONA M. BAYER, M.D.[2]

From the Department of Medicine, Stanford University School of Medicine, San Francisco, California

THE YOUNG DWARF and the young giant present problems which are humanly urgent, in that the physical peculiarity so greatly hampers social adaptation. These problems the physician is meeting with growing success and with a variety of methods. The present range of treatment possibilities was critically reviewed by Shelton in 1942 (1).

Some difficulties occur in evaluating treatment, because we know less about individual growth patterns of dwarfs and giants than we do about those of normal children. This article covers observations of from one to thirteen years on sixteen children with retarded growth and four children whose growth was accelerated.[3] Wherever possible, the data are presented from the viewpoint of a longitudinal study, as well as for their reflections on treatment. Although incomplete and tedious, longitudinal data on abnormal children are sufficiently scarce and suggestive to warrant being put on record.

METHOD OF STUDY

Records were analyzed throughout periods of treatment and control with regard to stature, linear growth rate, stature quotient, relative leg growth, bone age quotient, basal metabolism, intelligence quotient, and sexual development.

Absolute stature was plotted in centimeters for a visual comparison with normal (fig. 1). This chart is a summary of the stature data at a glance and can serve as a point of orientation for many of the other analyses of height. Normal curves are taken from Gray and Ayres (2); they were based on a group of children whose build may be regarded as optimum.

Linear *growth rate* was calculated according to the method outlined in a previous paper (3):

$$\frac{\text{Stature at end} - \text{Stature at beginning}}{\text{Average stature}} \div \text{period}$$

as decimal part of year. That is, if a child grew

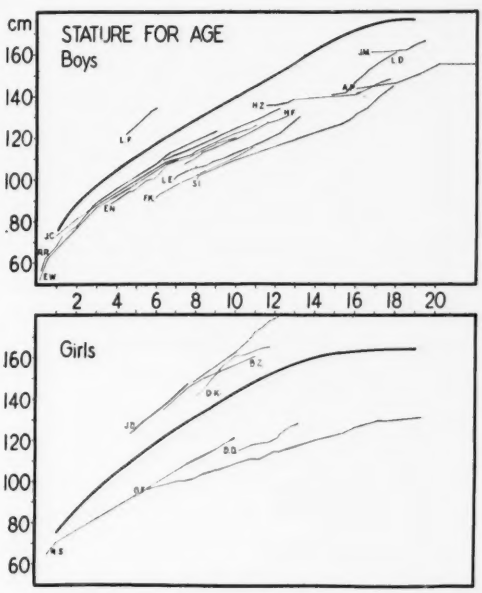

FIG. 1. Dwarfs and giants: individual stature curves can be compared with the heavy line showing average growth.

from 99 centimeters to 101 centimeters in three months, the rate would be:

$$\frac{101-99}{100} \times \frac{1}{0.25} = \frac{2}{25} = 8 \text{ per cent per year.}$$

Normal values for growth rate are taken from Gray (4).

[1] Read before the Twenty-Seventh Annual Meeting of the Association for the Study of Internal Secretions, Chicago, Illinois, June 12, 1944.

[2] Assistant Clinical Professor of Medicine.

[3] These children were referred to the Medical Endocrine Clinic by the Department of Pediatrics.

Stature quotient was calculated on the same principle as that conventional for intelligence quotients, namely, the observed stature on the subject is divided by the average stature for the same sex at the same age.

Relative leg growth. Before puberty, growth in stature is predominantly in the legs. Postpubertal growth is largely in the trunk. One way of demonstrating where growth is taking place is to compare the relation between sitting height and stature at successive intervals. If the percentage is dropping, the main growth is in the legs. Leg growth is the only kind of growth that can significantly affect height. Normal sitting height/stature indices are taken from Gray and Ayres (2).

Bone age quotient was calculated by reading every plate against suitable standards and then averaging the ages of all the bones. This bone age was then divided by chronological age. Knees and hands were read against Todd standards. The other standards used were Engelbach's.

Basal metabolic rates were calculated according to Boothby-Sandiford standards through age 16, Du Bois standards for later ages.

Intelligence quotients were calculated on the Stanford-Binet scales.

Results were evaluated by means of graphs. Treatment and control periods were scored for each factor as to whether there was increase, no result, or decline, compared to preceding levels. Subjectively, this has different import for dwarfs and giants, i.e., an increase in growth rate is a success in a dwarf, a defeat in a giant.

DWARFS

The status of the sixteen dwarfs at the beginning and end of observations is outlined in table 1. All the children were small, relatively well proportioned and without evidence of hypothyroidism or achondroplasia. Some had other complications, such as pituitary tumor or Madelung's deformity, but these complications are not pertinent to the study. Their dwarfism was of the type commonly called hypopituitary.

TABLE 1. DWARFS

NAME	SEX	FIRST OBSERVATION		LAST OBSERVATION		TREATMENT
		Age	Height	Age	Height	
		Years	*Cm.*	*Years*	*Cm.*	
L.B.	M	6.1	105.4	12.2	151.5	Thyroid
J.C.	M	1.0	73.7	11.0	127.2	None
D.D.	F	10.2	115.6	13.2	128.4	Growth hormone
L.D.	M	14.8	137.6	18.0	161.0	Growth hormone
L.E.	M	6.9	100.2	13.2	131.0	Thyroid; thyroid and growth h.; oreton
G.F.	F	5.6	96.5	19.4	131.0	Thyroid; growth h.; thyroid and growth hormone
H.F.	M	7.4	109.7	12.5	136.0	Thyroid
S.I.	M	8.0	101.3	18.3	146.0	Growth hormone
F.K.	M	5.9	91.4	13.1	126.2	Growth hormone
J.M.	M	16.8	162.6	19.5	166.4	Thyroid; growth h.
E.N.	M	3.8	88.9	10.0	121.7	None
A.P.	M	16.0	144.8	22.3	56.2	Thyroid
R.R.	M	0.3	56.6	9.0	135.0	Thyroid; growth h.; thyroid and growth hormone
R.S.	F	0.0	45.0	10.0	120.0	Thyroid; hyperthyroidism, spontaneous
E.W.	M	0.1	49.8	9.2	120.0	Thyroid
H.Z.	M	11.5	135.6	18.6	149.7	Growth hormone

Growth Patterns

Stature. By referring to figure 1, it can be seen that from the first observation dwarfs drop gradually further from the normal, which is well known, but after adolescence they gradually recover, which is a more encouraging feature not generally realized.

Study of *growth rate* explains the behavior of the stature. From sixteen charts made, three are presented to illustrate most of the points on which subsequent discussions are based (fig. 2). The general level of the growth rate may be depressed throughout, as in *G. F.*, or may be normal or even above normal, as shown in *L. D.* and *L. E.* Furthermore, all the dwarfs who were followed through the appropriate years, showed a late adolescent growth spurt,

sometimes of considerable magnitude (*L. D.*), and sometimes only a small flat hump (*G. F.*). This shifting of the growth spurt to the right is like the growth pattern of slow-maturing, normal children (5). The charts suggest that in long-time treatment of dwarfs. From this viewpoint our observations are disappointing. The greatest improvement in any case was 9 per cent in *L. D.* (fig. 2). Averaging together the differences between first and last readings in

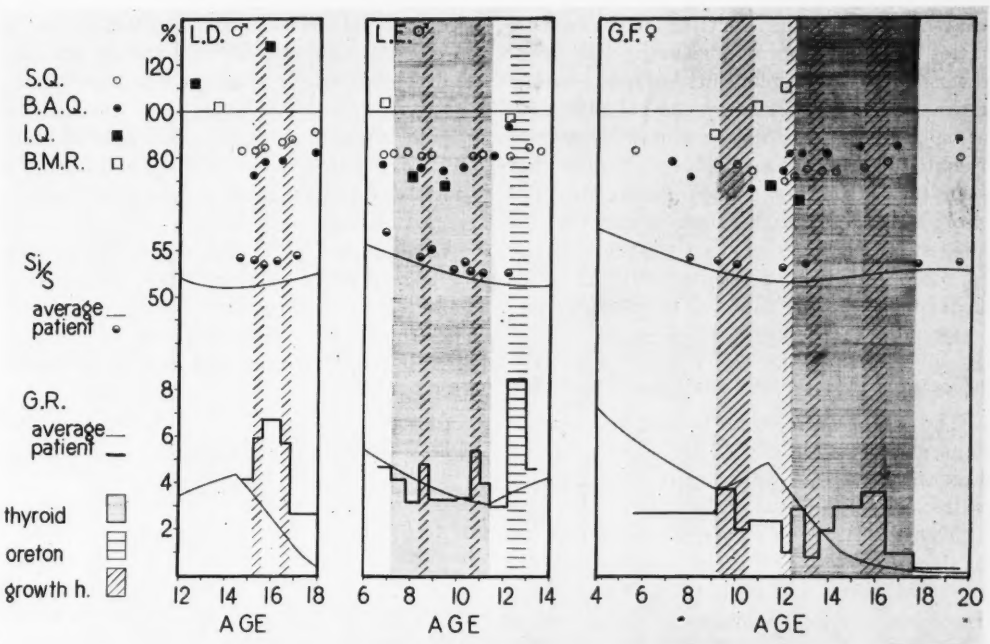

FIG. 2. Three dwarfs: graphic analyses of Growth Rate (G. R.); Sitting Height: Stature ratios (Si/S); Basal Metabolic Rates (B. M. R.); Intelligence Quotients (I. Q.); Bone Age Quotients (B. A. Q.); and Stature Quotients (S. Q.). All data are compared with the average; the four upper quotients vary around the normal 100 per cent line. Shadings indicate treatment periods. The arrow in chart G. F. indicates the age of menarche.

most dwarfs the main failure in growth impulse must be very early in life and that even fairly vigorous later growth never quite compensates for the original defect. The occasional large adolescent spurt looks almost as though there were sometimes one last effort to catch up to normal—if one should venture a teleological explanation.

Study of *stature quotients* also reinforces the visual impression of simple growth curves, i.e., stature quotient shows a drop up to the time of the child's late adolescence and then a gradual recovery. *L. D.*'s chart shows the late recovery; *L. E.* covers a long latent period; *G. F.* shows the whole cycle.

Clinically, the stature quotient is the most practical criterion of success or failure in the stature quotients, 8 cases showed an improvement of 3.1 per cent; the other 8 cases averaged a drop of 3.9 per cent. All but 2 cases received some treatment; the 2 untreated ones were among those showing a drop in stature quotient.

Bone age quotients parallel stature quotients in their tendency to fall lower before puberty, and then to recover. This is illustrated in all three cases and is similar to findings in some normal children in whom small early stature often goes with late maturation (6). Within the range of normal variations, however, the late-maturing child often grows as tall as or taller than his age peers, whereas dwarfs never catch up in stature, even though they reach complete skeletal maturity.

Curves of the sitting height/stature index show a fairly normal behavior of *leg growth*. Before adolescence, when the main growth is in the legs, the curves fall; they rise again after adolescence. This pattern is shown by all three children in figure 2. These curves also illustrate that most dwarfs have sitting height/stature ratios above the normal, meaning that most dwarfs are short-legged. Such a finding breaks down, somewhat, the conventional differentiation between proportionate and disproportionate dwarfs, since most dwarfs are thus seen to be somewhat disproportionate. The short-leggedness furthermore reinforces the suggestion made earlier, that the most serious failure in growth occurs very young. It is just in this early period that leg growth predominates most markedly.

Treatment with Thyroid and Growth Hormone

The *treatment schedule* for dwarfs was the same as outlined in 1937 (3). The first prescription was always a nutritionally adequate diet with the addition of vitamin concentrates.

Thyroid U.S.P. was given by mouth, started at 0.06 gm. daily and raised to tolerance, the eventual dose being usually 0.06 or 0.13 gm. Insomnia and weight loss were the symptoms which most often placed the ceiling on the thyroid dosage.

The growth hormones used were pituitary extracts[4] standardized at 10 units per cc. Three cc. were given three times weekly for periods of three months, or occasionally longer. The previous paper argues for the validity of such short periods (3).

The *results* of treatment were very difficult to evaluate; it is safer to talk about observations *during* treatment. Difficulties were greatest when treatment periods coincided with the patient's natural *adolescent growth spurt*. Since the adolescent growth spurt is a concept denoting a characteristic, limited period of acceleration in the growth rate, the most critical observations on the results of treatment during this period are those which note the behavior of the growth rate. For instance, seven of the sixteen dwarfs were followed through their adolescent spurt. When the behavior of the growth rate is observed during the whole span of these children's records, it is found that of 7 increases in growth rate under treatment, 6 coincided with the indigenous adolescent acceleration and 4 periods of drop in growth rate coincided with adolescent decelerations. This gives a coincidence of treatment with nature in 86 per cent of improvements and 57 per cent of decelerations, or a total coincidence of 71 per cent. Thus, it appears that the inherent growth tendency often supersedes the effect of any treatment and that the credit for growth spurts during treatment should sometimes be given to nature.

Examination of stature quotient, relative leg growth and bone age do not indicate such striking coincidence, nor is that to be expected since these other indices of growth are only indirectly related to the adolescent growth spurt itself.

Again, figure 2 exemplifies some individual observations during treatment.

L. D. This case was chosen particularly to illustrate the difficulty of interpreting results of treatment during the adolescent spurt. It is probable from the *growth rate* line that the first growth-hormone treatment period, which at the time seemed very encouraging, as viewed from later observations only looked so good because it coincided with the child's delayed adolescence. The second growth-hormone period apparently fell on the decline of the spurt and failed to halt the physiological decline.

L. E. This chart happily illustrates three dramatic improvements coincident with three treatment periods; two on thyroid and growth hormone, one on testosterone propionate.[5] In spite of his small stature and what must have been a previously depressed *growth rate*, the rate during our observations remains around normal and three times during treatment rises above normal. It is particularly interesting to study the *stature quotient* in this case because it illustrates that the good results on thyroid and growth hormone in regard to rate were not sufficient to bring the child significantly closer to normal height. During the period on testosterone, discussed below, the curve made a real rise. The sharp extended rise of the *bone age*

[4] Supplied through the courtesy of Ayerst, McKenna and Harrison Co.; E. R. Squibb and Sons; Parke Davis and Co.

[5] The androgen used was Oreton which was supplied through the courtesy of the Schering Corporation.

quotient curve can be interpreted as a maturation effect of the long-continued thyroid.

G. F. This is our longest-followed case, having been seen at fairly regular intervals from age 5 to age 19. The *growth rate* shows a generally depressed level and a late, small pubertal hump. Thyroid, given four separate times to this patient, three times improved the growth rate as compared to periods immediately preceding and following and once resulted in a relative depression. Growth hormone, when first given, seemed to exert a favorable effect, pushing the growth rate for a short time up to normal level. However, as the hormone was continued, the rate dropped down below its previous level and the second exhibition of hormone about a year later paralleled a clear-cut depression in growth rate. Thyroid and growth hormone together once paralleled a depression of rate and once gave an increase. The increase, however, was obviously riding on the inherent adolescent wave. This visual impression is confirmed by the appearance of the menarche shortly after the peak of the hump.

Despite reservations in interpretation, it was possible to make some decisions and to gather them together into figure 3. The main implications of this chart can be summarized.

Observations during *control periods* show a significant percentage of improvements, i.e., spontaneous rate rises and quotient rises. These improvements are usually reflections of post-adolescent recovery.

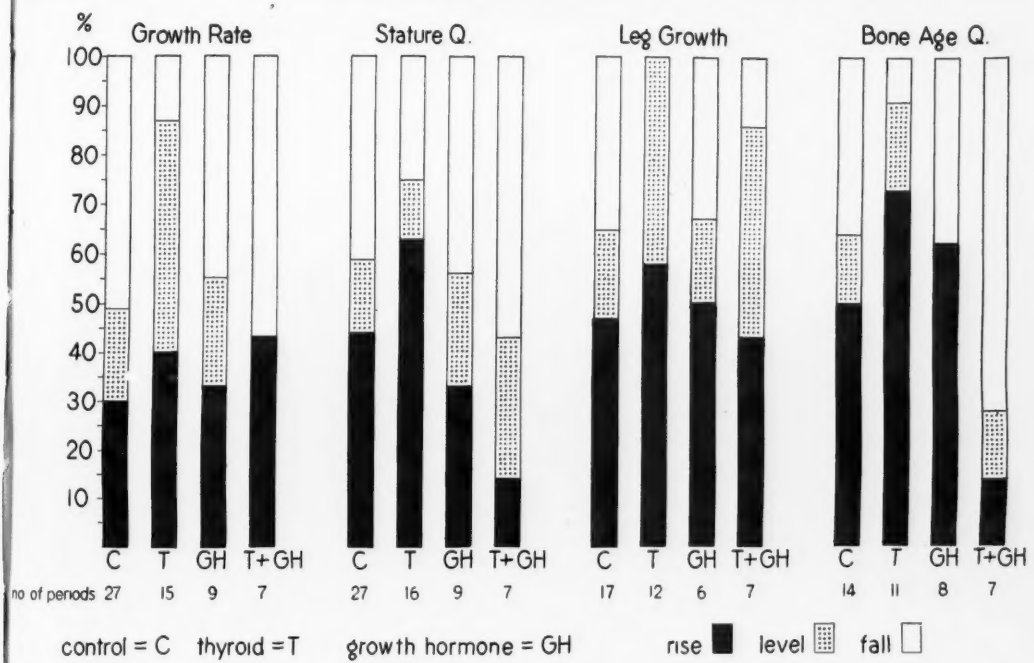

FIG. 3. Dwarfs: observations made during control periods and periods of treatment with thyroid, growth hormone, and their combination. % indicates the per cent of cases which showed a rise (improvement), level (no change), and a fall (deterioration).

The relative heights of the black columns indicate that stimulation of all phases of growth and development occurs most consistently during *thyroid* periods. The large proportion of instances in which the bone-age quotient rises during thyroid treatment illustrates the well-known tendency of thyroid to stimulate skeletal maturation, even more than it stimulates skeletal growth.

Observations during the administration of *growth hormone* indicate results which are less good than with thyroid.

Periods with *growth hormone and thyroid* together, however, show the highest proportion of improvements in growth rate. Even though this improvement is not enough favorably to influence the stature quotient, it still lends support to the generally accepted notion that thyroid and growth hormone reinforce each other. That the results of stature quotient do not corroborate the findings in growth rate is probably an accident of the times at which observations were made. As a practical concern, the improvements in *rate* before adolescence warrant continued trial of the combination of thyroid with new growth extracts as they are developed, even though the improvements so far have not been persistent enough to normalize a declining stature quotient.

TABLE 2. GIANTS

NAME	SEX	FIRST OBSERVATION		LAST OBSERVATION		TREATMENT
		Age	Height	Age	Height	
		Years	*Cm.*	*Years*	*Cm.*	
L.F.	M	4.4	121.7	5.7	132.3	None
B.K.	F	7.9	140.6	11.7	165.0	None
J.D.	F	4.7	117.5	12.7	175.1	Thyroid; thyroid and synapoidin
B.Z.	F	6.3	134.6	10.8	159.7	None

GIANTS

Observations were made on four young giants, all of them large, rapidly growing children without evidence of hypogonadism or of any endocrine syndromes occasionally associated with precocious growth. Three of these were girls who were followed through their adolescent growth spurt. Their height data are outlined in table 2. Their giantism was of the type commonly called hyperpituitary.

Growth Patterns

Stature. Going back to figure 1, it can be seen that the stature of giants, like the stature of dwarfs, at first moves further away from normal and then recovers. However, the return to normal is earlier in giants because, like tall normals, giants have an early adolescence.

Study of the other aspects of growth reinforces the impression that the growth of giants is just opposite to that of dwarfs. Figure 4 illustrates the characteristic high growth-rate level with a high early hump, followed by an early menarche. Not so clearly shown in the figure, but apparent from examining the charts of our other giants, are the following points. Before puberty the *stature quotient* rises; afterwards it falls. The *bone age quotient* parallels this phenomenon. Examination of *sitting height: stature ratios* shows that giants may be long-legged before adolescence, but return to average proportions or even achieve maturity with

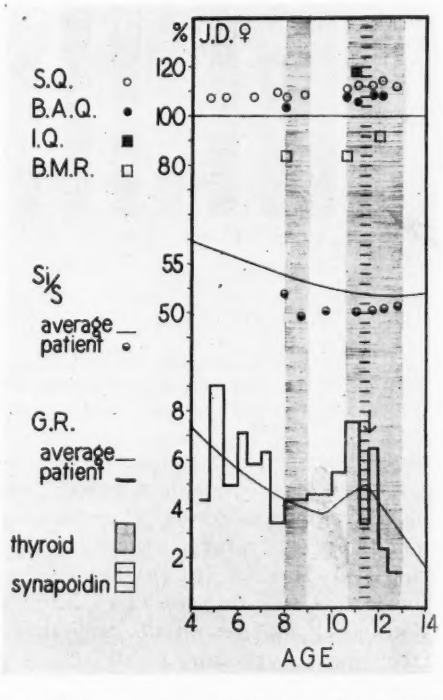

FIG. 4. Giant: graphic analysis of Growth Rate (G. R.); Sitting Height: Stature ratios (Si/S); Basal Metabolic Rates (B. M. R.); Intelligence Quotients (I. Q.); Bone Age Quotients (B. A. Q.); and Stature Quotients (S. Q.). All data are compared with the average; the four upper quotients vary around the normal 100 per cent line. Shadings indicate treatment periods. The arrow indicates the age of menarche.

relatively long trunks. The growth pattern of giants thus appears to be an exaggeration of the growth pattern of normal, early-maturing children (5,6).

Treatment with Thyroid

J. D., whose record is the substance of figure 4, is the only giant who was treated. Thyroid was given to tolerance because, in spite of her size, the child was clinically hypothyroid. Her basal metabolism was low; she was irritable; she had a poor attention span; her school work suffered from lack of concentration. On thyroid she was much more alert, happy and responsive. There was, however, no demonstrable influence on her growth rate, nor on her bone-age quotient, although in giving thyroid, it had been thought that the risk of a temporarily increased linear growth might be taken, in the hope of achieving earlier skeletal maturation. Observations made during administration of a gonadotropic hormone are discussed below.

SOME SPECIAL CONTRASTS BETWEEN DWARFS AND GIANTS

Data on basal metabolism, intelligence quotients, sexual development and the effect of sex hormones acquired more meaning when the findings on dwarfs and giants were pooled.

Basal Metabolic Rates

Basal metabolic rates were measured 21 times on 12 dwarfs, and 6 times on 3 giant girls. They are plotted on figure 5. Only one reading in each group was made during periods of treatment with thyroid, both of them falling below average, so that we can dismiss thyroid as a factor in the data.

The interesting finding illustrated in the chart is that 12 of the 21 readings on dwarfs lay above the average line, whereas 5 readings on giants fell below the line and none was above. There was no relation between the level of basal metabolic rate and the growth response to thyroid treatment.

One dwarf, *R. S.* (not illustrated), is especially noteworthy in that during the period of observation she developed a clinical hyperthyroidism following whooping cough, with a pulse rate of 120 and basal metabolic rate, +26 per cent. She was taking thyroid just before the whooping cough developed but did not resume medication. Although the basal metabolic rate later came back to normal, other symptoms of hyperthyroidism persisted. After the hyperthyroidism developed, the bones matured quite rapidly, the growth rate showed

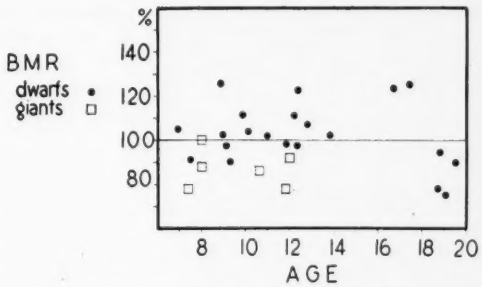

FIG. 5. Dwarfs and giants: distribution of Basal Metabolic Rates.

slight improvement, but the stature quotient recovered only a little.

Inasmuch as the data show so little consistency between basal metabolic rate, stature quotient or response to thyroid, it appears that the measurement of basal rate is not a good index of the growth-stimulating activities of the thyroid gland. Furthermore, the level of the basal metabolic rate is not necessarily a good criterion for the appropriateness of thyroid treatment.

A possible explanation of the occasional negative relation between growth rate and clinical metabolic rate may be offered. Indirect calorimetry measures only that fraction of the basal metabolism which involves oxygen consumption. But the metabolism of growth is largely the anabolism of protein and the synthesis of protein from amino acids does not directly require oxygen. The metabolism of too rapidly growing children might therefore use relatively less oxygen, that of too slowly growing children might use more, thus explaining the finding of the low rates in giants and high rates in dwarfs. Shock (7) notes a similar finding on five slow-maturing boys. It is as though, when the growth impulse is strong, the metabolism of growth takes priority

over the metabolism of maintenance in certain situations where there is not quite enough total activity to support both. Such an explanation is compatible with the occurrence of low basal metabolic rates in rapidly growing adolescents with simple goiter.

In giants, such low rates might fit in with clinical hypothyroidism (as in *J. D.*) or not (as in the other two girls), depending on whether or not the fraction of metabolism using oxygen was sufficient for all aspects of the body economy other than growth. Thyroid would be effective only where the total basal metabolism was insufficient. Conversely, where rates in dwarfs are high, thyroid might still sometimes

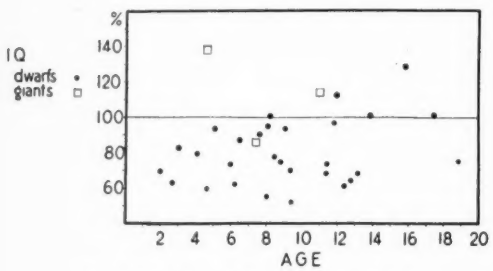

FIG. 6. Dwarfs and giants: distribution of Intelligence Quotients.

be effective in stimulating that portion of the metabolism which is involved in growth.

Intelligence Quotients

Intelligence quotients are plotted on figure 6. There are 29 tests on 14 dwarfs and 3 tests on 3 giants.

Only two dwarfs showed quotients above 110 and only one giant was mentally deficient. There is thus seen to be some relation, in our series, between mentality and stature status. This fits in with the normative finding that the rate of development in mind tends somewhat to parallel the rate of physical development.

Development of Secondary Sex Characteristics

At the last observation, seven of the sixteen dwarfs were retarded or deficient with regard to one or several aspects of physical sexual development and two of the four giants were accelerated.

Treatment with Sex Hormones

There is good rationale and increasing clinical support for the proposition that, given at the appropriate time, sex or sex-stimulating hormones may enhance deficient growth, perhaps discourage excessive increments. Two records in this series bear on the problem.

Testosterone propionate[5] was given, 10 mg. tablets three times daily, to the dwarf, *L. E.* (fig. 2). During the half year on testosterone he showed a distinct rise both in growth rate and stature quotient. There is no change in primary or secondary sex characters. He reported an increased feeling of well being.

Gonadotropic hormone[6] was given to the giant, *J. D.* (fig. 4), in an effort to terminate the adolescent growth acceleration and was superimposed on her thyroid treatment. One cc. was injected daily for five days, every 4 weeks for 4 months. Menarche followed a few days after the last injection. During the months of injection there was a sharp decline in growth rate, a standstill in an otherwise rising stature quotient, and a rise in bone age. Subsequent observations demonstrated that these injections were given at the beginning of what proved to be the decline of the adolescent growth spurt. However, the rate was actually lower than the rate in either the period which preceded or followed.

Thus, the administration of male sex hormone to a boy before the beginning of puberty paralled a clear-cut stimulus to linear growth; whereas gonadotropic hormone in a girl in mid-adolescence seemed to have a growth-inhibiting and maturing effect. This contrast might be fortuitous. It might reflect the earlier age at which the male sex hormone was given, when the hormone equilibrium is quite different from that in adolescence. It could also reflect a greater influence of male sex hormones on growth, of female sex hormones on maturation.

SUMMARY

Sixteen presumably hypopituitary dwarfs and four hyperpituitary giants were followed from one to thirteen years through periods of

[6] Synapoidin supplied by Parke Davis and Company was used.

treatment and control. Data are analyzed particularly from the longitudinal viewpoint.

Growth of dwarfs and giants shows contrasts similar to the contrasts that are seen between early- and late-maturing normals. In *growth rate* dwarfs tend to have low rates with late adolescent spurts, sometimes of considerable magnitude; giants have high rates with early spurts. In *stature* and *skeletal development* dwarfs fall further behind normal before their adolescence and recover gradually thereafter. Inversely, giants show pre-adolescent acceleration with subsequent return toward normal. When *sitting height* : *stature ratios* are noted, dwarfs tend to be short-legged; giants may be long-legged before adolescence, short- or medium-legged afterwards. *Basal metabolic rates* have a surprising distribution; many dwarfs have rates above normal; giants, below normal. It is suggested that this may be because, under certain circumstances when growth is more rapid, proportionately less oxygen is used during the basal metabolic test. Conversely, *intelligence quotients* tend to be low in dwarfs, high in giants. *Sexual maturation* tends to retardation and imcompleteness in dwarfs, to acceleration in giants.

Observations cover periods of treatment with thyroid, growth hormone, and sex hormones. The outstanding finding is that it is very difficult to evaluate treatment during adolescence, because *during adolescence in both dwarfs and giants, the natural growth pattern often supersedes the effects of any treatment.* In dwarfs, thyroid most consistently stimulated all phases of growth, showing the usual clear effect on skeletal maturation. Thyroid and growth hormone appeared to reinforce each other in stimulating growth rate. One growth-stimulating effect of sex hormone in a dwarf and one maturing effect in a giant encourage further trial with sex hormones and sex-stimulating hormones.

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Sexual Infantilism in Females[1]

Causes, Diagnosis and Treatment[2]

LAWSON WILKINS, M.D., AND WALTER FLEISCHMANN, M.D., Ph.D.

From the Department of Pediatrics, Johns Hopkins University School of Medicine, and the Harriet Lane Home of the Johns Hopkins Hospital

IN ANOTHER PAPER we (1) have discussed the failure of normal sexual development resulting from agenesis of the ovary and usually associated with a growth deficiency. There are a number of other conditions which cause failure of sexual maturation in the female. We shall attempt to classify them and shall present a number of cases of different types.

CLASSIFICATION

As shown in the accompanying table lack of development of the breasts, vagina and uterus and absence of the menarche may result either (a) from primary ovarian deficiency or (b) from a failure of the pituitary to produce the gonadotropic hormones which stimulate the maturation of the ovarian follicles.

Primary Ovarian Deficiency. We have reported four cases and have collected eight others from the literature in which there is substantial pathological evidence that the gonads never developed beyond the stage of the genital ridge which is found in the embryo of 5–7 mm. It is probable that in addition most, if not all, of the 26 clinical reports recorded by Turner (2), by Albright, Smith and Fraser (3), by Varney, Kenyon and Koch (4) and by Schneider and McCullagh (5) belong to this same group. In all 38 cases, except one reported by R. Meyer (6) and one reported by Schneider and McCullagh (5), the patient was short with a height between 50 and 59 inches. We believe that the stunted growth is best explained as an associated germinal or genetic defect and cannot be accounted for by an endocrine deficiency. In figure 1A one of our patients with ovarian agenesis is shown in comparison with a patient (fig. 1B) who was surgically castrated at the age of 12 years, 11 months.

It is probable that at times the development of the ovary may reach a more advanced stage than that of the genital ridge before becoming arrested. A number of cases in the literature are suggestive of this. Tronci (7) reported a 20-year-old girl, 58 inches tall, who had never menstruated. Her breasts were undeveloped but there was a scanty growth of pubic hair. Her ovaries were ovoid bodies 6×2.5 mm. in diameter. Whereas our patients showed no evidence of proliferation of the germinal epithelium, this patient's ovaries had down growths of germinal epithelium forming nests of cells in the stroma but no primordial follicles. Pich (8) described a girl 29 years of age who was 61.7 inches tall. Menstruation had never occurred; there was no development of the breasts nor any growth of sexual hair. Her right ovary measured 28×5×3 mm. and the left 30×4×2 mm. On section there were definite primordial follicles. Sellheim's (9) 21-year-old patient was 63.7 in. tall. She had never menstruated and showed no breast development. A scanty growth of pubic hair was present. The ovaries were spindle-shaped,

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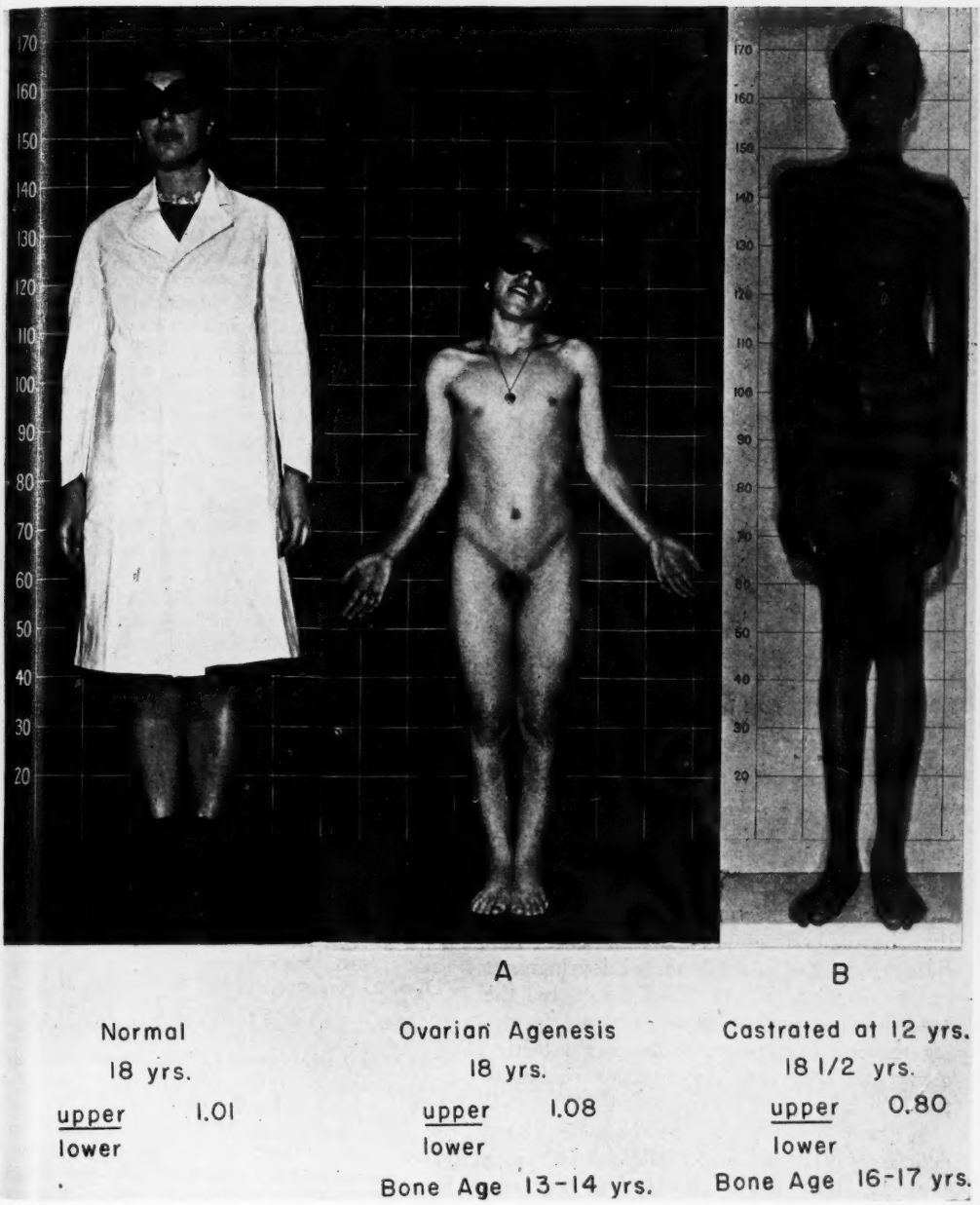

FIG. 1. Sexual infantilism due to primary ovarian deficiency.

measuring 25×7.5 mm. Sections showed numerous primordial follicles and some nests of cells resembling interstitial cells.

The cause of the arrested development cannot be determined from these pathological reports. No evidences of inflammatory or other destructive lesions were found in these four cases. Since assays of urinary gonado-

tropins were not made, it is impossible to decide whether or not failure of development may have depended upon deficiency of the pituitary gonadotropins. It should be pointed out, however, that in each instance the ovaries appeared very incompletely developed, even more immature than those of a normal newborn. Since it is unlikely that ovarian development depends upon pituitary stimulation much before the adolescent period, it seems improbable that the early failure of development in these cases can be attributed to pituitary deficiency. Certainly the ovaries were far different from those observed by us in a patient with a selective deficiency of the pituitary gonadotropins whom we shall discuss later. There remains, however, the possibility that in these patients the ovaries may have reached a stage of preadolescent development and then, failing to receive the normal pituitary stimulus, may have retrogressed and atrophied.

It is theoretically possible that in some cases primary ovarian deficiency may result from a destructive lesion or a degenerative process. The final effects of such deficiency upon skeletal growth and other body functions would probably depend upon the age of the patient when it occurred. We have been unable to find any reports in the literature in which there was clear-cut pathological proof of ovarian destruction or degeneration. Albright, Smith and Fraser (3) in their paper on "A Syndrome Characterized by Primary Ovarian Insufficiency and Decreased Stature" mention that they have observed two patients of normal stature, who had infantile sexual development but an *increased* F.S.H. They designated the condition "premenarchial menopause," implying thereby that prior to the menarche the ovary had undergone degeneration similar to that which occurs at the menopause.

We have not recognized any cases of ovarian deficiency occurring before adolescence which could be attributed to spontaneous degeneration or destruction of the ovary. However, we are able to report a patient in which the same condition was produced by surgical castration at the onset of adolescence.

E.L. (*H.L.H. 43074*) (see fig. 1B) grew and developed normally during her early years but always was inclined to be tall and thin. At the age of 9 years her height was 51 inches (the average height for 10 years). At 11 years it was 57.6 inches (the average for 12 years). In the next 23 months she grew 5.4 inches attaining a height of 63 inches (the average for 16 years). At the age of 12 years and 11 months, it was stated that her breasts were "well developed for her age" and that the external genitalia were "apparently normal for her age." No mention was made in the hospital note as to whether pubic hair was present. Menstruation had not occurred. At that time she developed sudden severe pain in the left lower quadrant and difficulty in voiding. A movable mass was felt in the lower abdomen. A laparotomy was performed on the Gynecological Service and both ovaries were found to be cystic, each measuring 8×6 cm. The left ovary had a very long pedicle and was freely movable. The uterus was "very small, but normal in appearance and consistency." Both ovaries were removed and on section showed dermoid cysts. One of the cysts contained bone, some sebaceous material and a small amount of short, black hair.

Following the removal of the ovaries in October, 1937, the patient grew 1 inch in the next two years, measuring 64 inches in December, 1939, and another 1½ inches in the following two years, reaching a height of 65½ inches in October, 1941. She continued to grow and was 66.7 inches in June, 1943, at the age of 18½ years. Following operation her breast development had disappeared but pubic hair had continued to grow. Menstruation never occurred. Figure 1B shows her appearance at 18½ years compared to a patient with complete ovarian agenesis. Her measurements were:

	Patient	*Average for height*
Age:	18½ years	
Height:	66.7 in.	
Weight:	106.5 lbs.	130 lbs.
Span:	74.0 in.	68.1 in.
Upper segment: (pubis to vertex)	29.6 in.	33.5 in.
Lower segment: (pubis to soles of feet)	37.1 in.	33.2 in.
Ratio $\frac{\text{upper}}{\text{lower}}$	0.80	1.01
Head (circumference)	20.9 in.	21.9 in.
Chest:	28.2 in.	31.0 in.
Abdomen:	25.0 in.	25.7 in.

The span and the lower skeletal segment were each 4 inches longer than the average for the height. These are the typical "eunuchoid" proportions. Her trunk was slender and straight without flaring of the pelvis or constriction of the waist. The osseous development was similar to that of a normal 16- to 17-year-old girl. X-ray plates of the bones showed complete fusions of the epiphysial lines at the elbow, the os calcis, the hip and the metacarpals. The epiphyses at the knee were almost completely fused. Those at the ankle, shoulder and wrist were still open. Dental development was well advanced, four wisdom teeth being present. The nipples, areolae and mammae were entirely undeveloped although there was very slight padding of fat in the breast region. There was an abundant growth

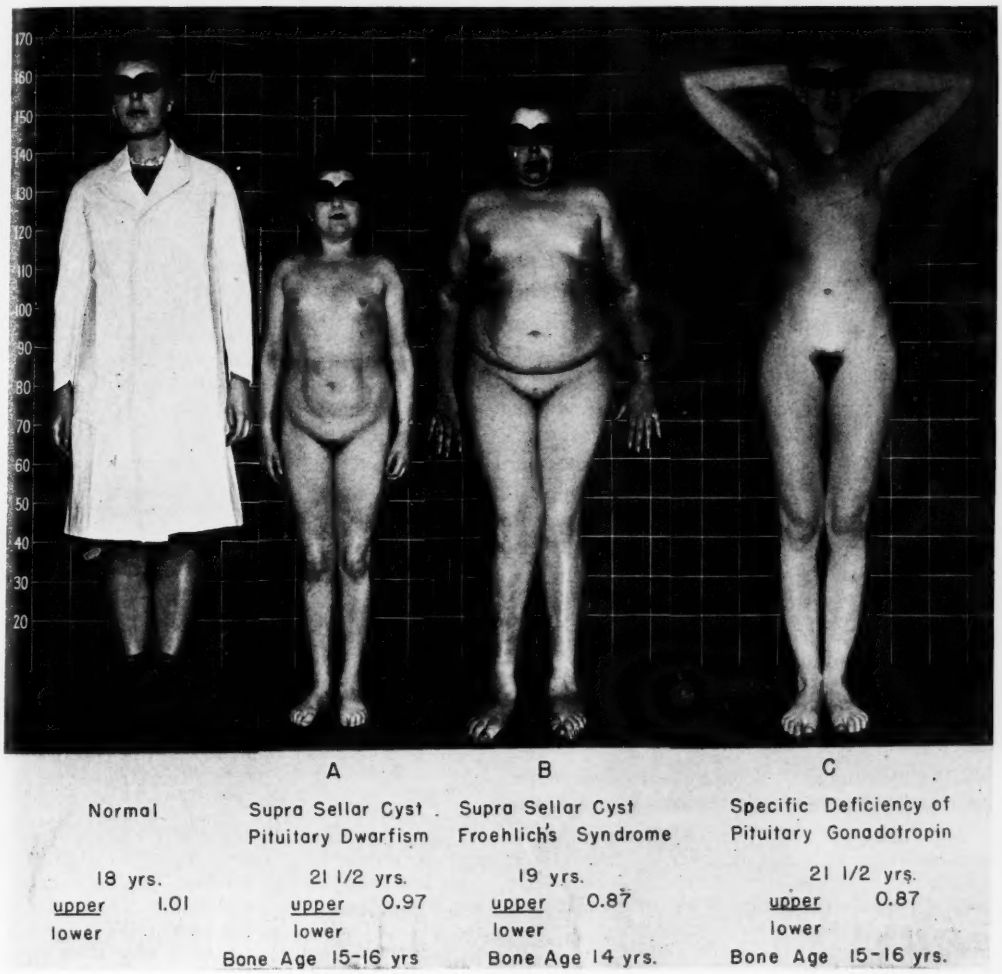

FIG. 2. Sexual infantilism due to pituitary deficiency.

of pubic and labial hair showing normal female distribution and a slight growth of axillary hair. The labia majora and minora were undeveloped. The clitoris was of normal size. The hymen was present. The vaginal outlet was quite voluminous and covered by an atrophic-appearing mucosa. The cervix was but a small nubbin. The uterus and cervix made up a mass which felt no larger than the end of one's thumb. A uterine sound was passed through the cervical canal without much difficulty to a depth of 3.5 cm. Biopsy of the mucous membrane from the cervix and the vagina showed a prepubertal type of epithelium.

An assay of the *urinary gonadotropins showed* 15 R.U. per 24 hours by the Delfs (10) method, whereas the normal is 10 to 20 R.U.

The excretion of 17-ketosteroids was 2.3 mg. per day.[3] The normal adult female excretes from 3 to 9 mg. per day.

Comment. Although menstruation had never occurred the patient's breasts had already begun to develop before operation, giving evidence that estrin was being produced by the cystic ovaries. Castration at this stage of adolescence led to typical eunuchoid development.

[3] The determinations of the 17-ketosteroids were made by Dr. Elizabeth G. Frame, of the Brady Urological Institute, who used the method published recently by her (*Endocrinology* 34:175. 1944).

Ovarian Deficiency Secondary to Pituitary Deficiency. The classical examples of sexual infantilism due to pituitary insufficiency are the hypopituitary dwarfs. Characteristically, hypopituitary dwarfs show not only lack of development of the breasts, vagina and uterus but absence of pubic and axillary hair, indicating that there is deficiency not only of estrin but also of the adrenal androgens. These cases usually result from a generalized deficiency of all the pituitary functions and usually are not associated with gross destructive lesions which can be readily demonstrated clinically. It must be recognized, however, that pituitary disorders may cause clinical pictures which differ from those of the typical pituitary dwarf and yet lead to sexual infantilism. We have selected as examples of sexual undevelopment due to pituitary disturbance two patients in whom definite lesions were demonstrated in the region of the sella turcica. They differ considerably from one another. One patient was markedly dwarfed although biochemical studies did not conform in all details with the criteria suggested by Fraser, Albright et al. (11, 12) for the diagnosis of "panhypopituitarism." The other patient was not dwarfed but had eunuchoid proportions and obesity of the sort associated with the so-called Froehlich's syndrome.

B.S. (H.L.H. A 30135 (fig. 2A) first came under our observation at the age of 21 years, 8 months. Her growth and development were thought to have been entirely normal up to the age of 5 years. At that time she fell down a flight of steps striking her head and was semiconscious for 24 hours. Following this, her appetite decreased and her growth and development slowed up coming almost to a standstill. She began to have frequent attacks of vomiting. At the age of 7–8 years frequent headaches began to occur. She failed to lose her deciduous teeth; her skin became somewhat dry and rough, and her hair dry and brittle. At the age of 10½ years her height was 45 inches (the average for 6¼ years) and her bone-age was 6–7 years. Between the ages of 11 and 13 years she was treated with various pituitary preparations both orally and intramuscularly and was also given desiccated thyroid without any decided effect. In 1934, at the age of 15 years, spasticity developed on the left side of the body and the vision of the left eye became blurred. There were polyuria and polydipsia. Ophthalmoscopic and x-ray studies led to a diagnosis of suprasellar cyst. Dr. C. H. Frazier of Philadelphia performed a craniotomy, found the cyst and aspirated 5–6 oz. of fluid. A decompression was left. A second aspiration was done followed by radiation of the region. All neurological and ophthalmological signs and symptoms cleared up and have never returned.

Following the operation she was treated by Dr. Leonard Rowntree and others with various pituitary preparations, including Armour's "Growth Complex," thymus preparations, chorionic gonadotropins ("Antuitrin S") and thryoid. The details of treatment are not available at the present time but therapy caused no convincing acceleration of growth or development. The patient was first seen by us at the age of 21 years, 6 months. During the year prior to this, her breasts had enlarged slightly and a little pubic hair had grown. Menstruation had never occurred. It is not certain whether the patient ever had been given estrogens. She thinks that she may have taken an ovarian preparation for a short period but this cannot be confirmed. She had discontinued thyroid two months previously without any noticeable change in her condition. Her appearance is shown in figure 2A and measurements follow.

	Patient	*Average for height*	*Average for age*
Age:	21 yrs., 8 mos.		
Height:	53.5 in.	Height-age: 10¼ yrs.	64 in.
Weight:	78.5 lbs.	65.4 lbs.	117.5 lbs.
Span:	55.2 in.	53.1 in.	64.8 in.
Upper segment: (pubis to vertex)	26.3 in.	26.9 in.	33.5 in.
Lower segment: (pubis to soles of feet)	27.2 in.	26.6 in.	31.9 in.
Ratio $\frac{\text{upper}}{\text{lower}}$	0.97	1.01	1.01
Circumference of head:	20.5 in.	20.7 in.	21.0 in.
Chest at nipples:	30.2 in.	25.3 in.	31.0 in.
Abdomen:	25.6 in.	22.0 in.	25.7 in.

The patient was 11 inches below the average height and was the size of an average 10¼-year-old girl. Her span and lower skeletal segment were only slightly longer than the average in relation to her height. Her features and her hands and feet were small and dainty. In both her appearance and her manners there was a curious mixture of adult sophistication and childishness. Mentally she was intelligent and alert. The trunk was of medium build and the contours though somewhat feminine were not those of a mature female. Subcutaneous fat was not excessive and was normally distributed. Her skin was of good color, warm, soft and moist, and the hair of the head was abundant and glossy. All of the permanent teeth except the third molars were present and normally formed. The osseous development was slightly less than normal for 16 years. The epiphyses of the os calcis and those at the elbow were completely fused while the epiphysial lines were only partly closed at the hips, phalanges, metacarpals, knees, ankles and wrists. There were small swellings in the breast region and a definite but poorly demarcated zone of glandular tissue about 5 cm. in diameter was present. The areolae measured 2.3 cm. in diameter and were but little pigmented. The nipples were small but slightly erectile. There were a very few scattered coarse, dark hairs over the pubis and the labia majora.

Both the labia majora and minora were undeveloped and unpigmented. The clitoris was small. On examination under anesthesia the vulval outlet barely admitted one finger. The vaginal mucosa was red and smooth, and biopsy showed a prepubertal type of epithelium. The cervix was only a small nubbin. On bimanual examination both rectally and vaginally neither the uterus nor adnexa could be felt.

Ophthalmoscopic and perimetric examination showed normal fundi and visual fields. There was mild hyperopia with astigmatism. Neurological examination was negative.

The examination of the urine gave normal findings; the output was normal in volume and specific gravity. Chemical examination of the blood serum showed N.P.N., 27 mg.%; Ca. 10.2; mg.%; phosphorus, 3.9 mg.%; cholesterol, 313 mg.%. The basal metabolic rate was 750 calories per 24 hours, which was 21% below normal according to the Boothby-Sandiford Standard. The glucose tolerance curve obtained after the oral administration of 1.75 gm. glucose per kg. of body weight was not abnormal: fasting, 87 mg.%; ½ hour, 139 mg.%; 1 hour, 112 mg.%; 1½ hours, 117 mg.%; 2 hours, 129 mg.%; 3 hours, 83 mg.%. The response to hypoglycemia tested by injection of 0.1 unit insulin per kg. was normal: fasting, 92 mg.%; 20 min., 61 mg.%; 30 min., 64 mg.%; 45 min., 99 mg.%; 80 min., 87 mg.%. Unfortunately, the urinary preciptate for assay of the gonadotropins was lost and this was not discovered until after treatment with estrogens was begun. The excretion of 17-ketosteroids was 4.1 mg. in 24 hours.

Comment. This patient had a cyst in the suprasellar region, which probably led to disturbance of some of the pituitary functions rather than a destructive lesion of the pituitary gland or a generalized deficiency of its activities. The patient differed from the cases of panhypopituitarism described by Albright in not showing a decreased response to hypoglycemia induced by insulin and in having a moderate output of 17-ketosteroids and a slight growth of pubic hair. It is not certain whether the slight development of the breasts resulted from a feeble activity of the patient's pituitary or whether it was due to therapy previously administered.

H.C. (*H.L.H. A 22883*) (fig. 2B) was first seen at the age of 19 years. Her early growth and development had been normal. During childhood she was not considered especially stunted in her height but had failed to show an adolescent spurt of growth. However, during the past year she had grown more rapidly, increasing 2 inches in height. She had begun to gain weight excessively at 6 years of age and her weight had increased steadily since. She had never menstruated and her breasts and pubic hair had not developed. For years she had had polydipsia and polyuria. She had had no headaches, visual disturbances or neurological symptoms. Her appearance is shown in figure 2B at the age of 19 years, when her measurements were:

	Patient	*Average for age*	*Average for height*
Height:	60 in.	64 in.	"Height-age" 13¼ yrs.
Weight:	152 lbs.	117 lbs.	92.6 lbs.
Span:	62.4 in.	64.8 in.	60.2 in.
Upper segment: (pubis to vertex)	26.8 in.	32.1 in.	29.9 in.
Lower segment: (pubis to soles of feet)	33.2 in.	31.9 in.	30.1 in.
Ratio $\frac{\text{upper}}{\text{lower}}$	0.87	1.01	0.99
Head circumference:	21.8 in.	21.9 in.	21.2 in.
Chest:	39.0 in.	30.9 in.	28.1 in.
Abdomen:	35.4 in.	25.6 in.	23.9 in.

The girl was only 4 inches below the average height. However, the span was 2.2 inches and the lower skeletal segment 3.1 inches greater than the average for height, a disproportion suggestive of eunuchoidism. She was 60 lbs. overweight, had a pendulous abdomen and folds of fat over the chest. The bone age was about 14 years. The trochlea, capitellum, epicondyles of humerus and head of the radius had fused but all other epiphysial lines were open. Four 12-year molars were present. There were pads of fat in the region of the breasts but no mammary tissue was palpable. There was no pubic, labial or axillary hair. The external genitalia were those of a young child. A very small uterus could just be felt. The ovaries were not palpable.

Stereoscopic plates of the skull showed an irregular area of calcification in the midline just above the sella turcica. The sella itself was quite small but there was no evidence of bony destruction. Ophthalmoscopic examination revealed no abnormalities and the visual fields were normal. The daily output of urine ranged between 3000 and 4000 cc. with a specific gravity of 1.005–1.006. Assay of urinary gonadotropins by the method of Heller and Heller showed an excretion of less than 5 R.U. in 24 hours. The daily output of 17-ketosteroids ranged between 0.3 and 0.5 mg.

Comment. The obesity, sexual undevelopment and diabetes insipidus constitute the Froehlich's syndrome which usually is caused by a lesion in the hypothalamus rather than a primary pituitary disorder. The gonadotropic activity of the pituitary is inhibited secondarily in such lesions. In this case the absence of pubic hair and the low 17-ketosteroid excretion indicated also a low androgenic activity of the adrenals such as is found in true "panhypopituitary dwarfism."

It seems probable that there may occasionally occur a *selective deficiency of the pituitary gonadotropins* without evidence of diminution of other pituitary functions. Albright (3) observed one young woman of normal height who showed lack of development of the female sexual characteristics but had normal amounts of pubic and axillary hair and a moderate excretion of the 17-ketosteroids. Unlike patients with primary ovarian deficiency, her urinary gonadotropic excretion was not increased. We have seen a similar case.

J.O. (*J.H.H. No. 285399 Gyn.*) (fig. 2C). The patient's early development had been normal. During early childhood she had always been short for her age but she had a spurt of growth between the ages of 19½ and 20½ years. Between the ages of 15 and 16 pubic hair had appeared and had increased gradually since. About the same time a slight growth of axillary hair had appeared. Her breasts had never developed and menstruation had not occurred. Her appearance at the age of 21½ years is shown in figure 2C. At this time her measurements were:

	Patient	*Average for height*
Height:	65 in.	
Weight:	109.2 lbs.	122.2 lbs.
Span:	67.0 in.	66.0 in.
Upper segment: (pubis to vertex)	30.3 in.	32.6
Lower segment: (pubis to soles of feet)	34.7 in.	32.4 in.
Ratio $\frac{\text{upper}}{\text{lower}}$	0.87	1.01
Head circumference:	22.0 in.	21.9 in.
Chest:	29.0 in.	31.9 in.
Abdomen:	26.0 in.	25.7 in.

This patient showed eunuchoid proportions with a span 1.0 inch and a lower skeletal segment 2.3 inches greater than the average for her height. Her trunk was slender, the chest was "boyish" but there was flaring of the pelvis and a feminine waist. She was 13 lbs. below the average weight for her height. Her bone age was 15–16 years. The epiphysial lines at the elbow, hip and os calcis had closed completely. Those of the metacarpals, shoulder and ankle were partly united, while the epiphyses at the wrist were widely open. The bones showed moderate rarefaction. One wisdom tooth had erupted. The breasts were entirely infantile with tiny nipples and unpigmented areolae measuring 1.5 cm. in diameter. No mammary or duct tissue was palpable. There was a moderately abundant growth of coarse dark hair over the labia and pubis and a moderate growth in each axilla. There was very little development of the labia majora and the labia minora were quite small. The clitoris was normal. The outlet admitted two fingers only with difficulty. The vaginal wall was very smooth. Vaginal smear and biopsy showed lack of estrin effect. The cervix was small, about the average size for a 7- or 8-year-old girl. The ovaries could not be felt. X-ray study of the skull showed no abnormalities and the ophthalmoscopic findings were normal. There were no evidences of diabetes insipidus. Assay of the urine for gonado-

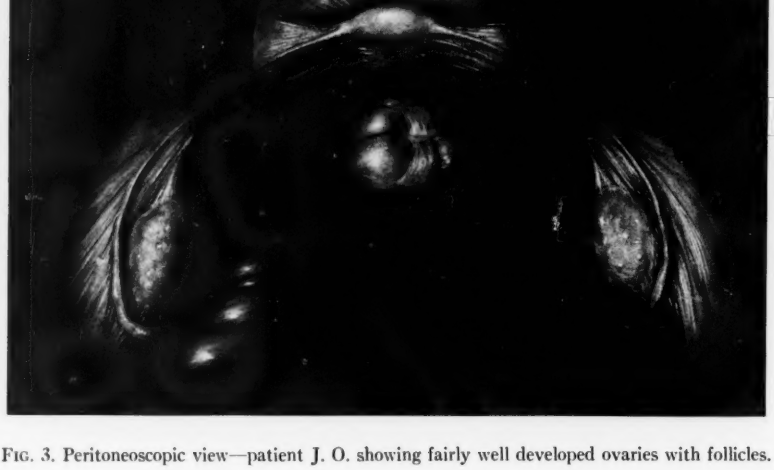

FIG. 3. Peritoneoscopic view—patient J. O. showing fairly well developed ovaries with follicles.

tropins by Dr. Seegar-Jones by the method of Delfs (10) using the uterine weight of the immature rat showed less than 6 R.U. per 24 hours. An assay by Dr. Klinefelter (13) by his method using the uterine weight of the immature mouse gave negative results on a morning specimen. Assays for 17-ketosteroids showed 4.4–5.8 mg. per day.

Exploratory laparotomy was refused but a peritoneoscopic examination was made by Dr. TeLinde. A very small uterus about 2 cm. in width was seen. The fallopian tubes were extremely thin but appeared longer than the average. On each side an ovary was visible which appeared to be of normal adult size. It was white and glistening. There appeared to be small follicles on the surface. No corpora lutea were seen. Figure 3 is an artist's drawing of the appearance through the peritoneoscope.

Comment. The normal height and eunuchoid proportions, the abundant pubic hair and the nearly normal excretion of 17-ketosteroids, at first suggested that this was a case of primary ovarian deficiency. However, the demonstration of a low output of urinary gonadotropin suggested that the deficiency might be primarily pituitary rather than ovarian. This was confirmed by the findings of ovaries of normal size showing follicle formation. The follicles were apparently incapable of secreting estrin. There was no evidence of any other dysfunction of the pituitary except the gonadotropic activity.

OTHER CAUSES OF INCOMPLETE SEXUAL MATURATION

It is not the purpose of this paper to discuss all the causes which may lead to a failure or delay of normal sexual maturation and the establishment of normal cycles of menstruation. It is important to recognize that there are wide variations in the time and patterns of adolescence. Not infrequently there are maladjustments in the balances of follicle-stimulating, luteinizing and other hormones resulting in menstrual irregularities and minor disturbances of sexual development. Other endocrine dysfunctions, particularly hypothyroidism, are frequently blamed for such disturbances, often without substantial proof.

Adrenal disorders may cause alterations in the normal pattern of female sexual development. When Cushing's syndrome occurs before the age of puberty the breasts and sexual organs remain undeveloped and the lack of cornification of the vaginal mucosa indicates a deficiency of estrin. Pubic and capillary hair develop due to the presence of androgens. The reverse is seen at times in Addison's disease (corticoadrenal insufficiency) in which sexual hair may be lost although normal menstrual cycles and breast development may persist (3, 14). Embryonic hyperplasia of the androgenic elements of the adrenal leads to pseudohermaphroditism in the female. The ovaries are present but immature. The uterus, tubes and breasts remain infantile. The phallus hypertrophies, the urogenital sinus develops along the male pattern and the excessive production of androgen causes precocious growth of sexual hair.

Untreated thyroid deficiency of marked degree causes a prolonged delay in sexual development just as it causes delays in growth, osseous and dental development. Eventually even the untreated cretin usually acquires adult sexual characteristics even though entirely normal sexual maturation may not occur. In endemic cretinism the menarche is usually delayed, menstrual bleedings are scanty and irregular. However, complete absence of the menarche is not rare nor the occurrence of abnormal profuse bleedings. Pregnancy with delivery at term has been observed not infrequently in cretins (DeQuervain and Wegelin, 15).

We have seen one cretin (*G.S., H.L.H. 34878*) who was entirely untreated until the age of 24 years. Her breasts had begun to develop at 16–17 years and pubic hair appeared at 22 years, at which time she first menstruated. Since then she had menstruated about once every 6 months. On some occasions the bleeding had been excessive but had never continued longer than three days. At 24 years her breasts, were fairly prominent, slightly pendulous about 12 cm. in diameter. They contained considerable fat but only a moderate amount of glandular tissue. The nipples were erectile and well developed and the areolae were deeply pigmented. There was a sparse growth of pubic hair. The vaginal mucosa showed adult rugae. The uterus was about two-thirds the adult size and the cavity measured 8.5 cm. in length. On palpation the left ovary was felt to be of normal size and the right about half the average size. Biopsy showed a non-secretory type of

endometrium. Gonadotropic assay (Fleischmann) on the immature rat ovary was negative, indicating an excretion of less than 25 rat units per liter.

Another cretin (*T.E.A. 74071*) was seen in the Gynecological Department at the age of 17 years and 9 months. Although the diagnosis of cretinism had been made in infancy, she had received very little thyroid therapy and that only occasionally at long intervals, so that she still presented an extreme picture of cretinism. She had had one menstrual period of three days months later, and after 9 months another prolonged period of menorrhagia required a repetition of the curettage.

DIAGNOSIS

In the table are shown some of the principal findings which may be of value in differentiating the various types of sexual infantilism. In the light of our present knowledge one cannot be as dogmatic in diagnosis as this table indicates and a number of the subjects merit special discussion.

Causes of Sexual Infantilism	Menstruation Development of Breasts Vagina Uterus	F.S.H. Excretion	Sexual Hair and 17-K.S. Excretion	Skeletal Development			Frequently Associated Defects
				Height	Lower Extremities	Epiphysial Development	
A. *Primary Ovarian Deficiency*							
1. Failure of Development	0	+	+	Usually short	Short?	Slight delay	Webbed neck Coarctation aorta Hypertension Osteoporosis Eye defects Congenital deafness
a. Agenesis							
b. Arrested Development							
2. Degenerative or Destructive Lesions (or surgical castration)	0	+	+	Usually tall	Long	Slight delay?	
B. *Pituitary Deficiency with Secondary Hypoovarianism*							
1. Generalized Pituitary Deficiency (Dwarfism)	0	0	0	Short	Normal ratio	Marked delay	Other signs of pituitary deficiency
2. Froehlich's Syndrome	0	0	0 or ±	Variable	Long?	Slight delay?	Diabetes insipidus; obesity
3. Selective Deficiency of Pituitary Gonadotropin	0	0	+	Tall	Long	Slight delay	

at 17 years. No further bleeding occurred until 12 days before admission when a profuse menorrhagia began and persisted to the point of causing marked anemia. At the time of admission, her breasts were both large and firm, the left much larger than the right. The duct structure especially seemed to be hypertrophied. The nipples and areolae were of adult size. The labia majora were normal. The labia minora were greatly elongated and hypertrophied and hung down like a scrotum, measuring 6–7 cm. in length. The vaginal outlet admitted one finger. The cervix was small, the uterus of normal size. Both ovaries were small, about 2 cm. in diameter. Biopsy showed endometrium of the non-secreting type. After uterine currettage bleeding stopped. Another 7-day period of menstruation occurred 3

Assay of Urinary Gonadotropins. Theoretically, the finding of an increased excretion of follicle-stimulating hormone would be expected in primary ovarian deficiency and a decreased excretion in hypo-estrinism due to pituitary deficiency. A biologic assay of this sort requires very careful standardized technic and is of value only when carried out by workers especially experienced in its use. Since the test was first introduced by Zondek in 1930 (16) it has been modified by various workers (Heller and Heller, 17; Levin and Tyndale, 18; Varney and Koch, 19; Klinefelter et al., 13) in attempts to improve its quantitative accuracy and make it applicable for clinical diagnosis. A great variety of units and methods have been used by the different workers for the assay of gonadotropins. As Delfs has pointed

out, "The existence of so many methods is evidence in itself that none has been particularly satisfactory." The increase in the weight of the uteri of an immature rodent seems to be the most satisfactory endpoint in the assay of gonadotropin. Vaginal cornification and uterine weight methods can be employed together, the first being used as a check on the second (D'Amour and D'Amour, 20). Whereas some authors use rats (Delfs, 10; D'Amour and D'Amour, 20), others feel that the uterine weight response in the immature albino mouse to gonadotropin is much more uniform and more sensitive than in the rat (Evans, Hines, Varney and Koch, 21). In determining which fraction of the gonadotropins is being excreted the use of hypophysectomized rats and a combination of methods using both changes in the weight of the uteri and changes in the weight and histological appearance of the ovaries may become necessary (Leathem and Levin, 22). Due to these variations in the technic it is difficult to compare the findings of different authors who have studied different clinical conditions. The diagnostic value of such a test can be established only when the results have been correlated in a large number of different clinical conditions in which the diagnosis has been established beyond doubt. A combination of methods may often be employed with advantage (Rakoff, 23).

The Massachusetts General Hospital group of workers have had a large experience in the clinical application of the assay to endocrine diagnosis and have published the details of their modification of the Heller and Heller technic (13) by which they can assay decreased as well as normal and increased amounts of the hormone. They found that normal males and females between 20 and 45 years excrete 6.6 to 53 mouse units per 24 hours. Patients with hypo-estrinism excreting larger amounts of F.S.H. were considered to have primary ovarian deficiency and those having a decreased excretion were believed to be lacking in the production of the hormone by the pituitary. They found a number of patients with hypo-estrinism who excreted normal amounts of F.S.H. and suggested that in these instances there was a "complete lack of release of luteinizing hormone" and that pure F.S.H. will not produce estrogen except in the presence of small amounts of L.H.

In studying the problems of sexual infantilism or of abnormalities in the onset of puberty we do not believe that at the present time the results of assays of urinary gonadotropins can be interpreted with absolute certainty, although there are some basic data available on the excretion of F.S.H. during the various stages of normal puberty and in cases of early and late adolescence (Nathanson et al., 24; Catchpole et al., 25). As Varney, Kenyon and Koch have suggested, it is possible that in some cases of delayed adolescence, high excretion of gonadotropins might indicate an increased activity of the pituitary gland in an effort to stimulate maturation of the ovaries. It need not necessarily mean that the ovaries are lacking or are incapable of stimulation. On the other hand, it is not certain that absence of the ovaries will result permanently in excessive excretion of gonadotropins. Perhaps our hesitation to base too much diagnostic importance on the test as carried out in our laboratories has been enhanced by our own experience in the study of 3 cases of ovarian agenesis and one of prepubertal castration. These cases illustrate the difficulties whether they are due to technic or interpretation. A girl (*E.L.*) who was surgically castrated at the age of 12 years excreted only 15 rat units per day at the age of 18 years with the Delfs (10) technic using hypophysectomized rats. Normal women excreted 10–20 units per day. Another patient (*L.M.*) proven to have ovarian agenesis excreted less than 6 R.U. before treatment and 14 R.U. after withdrawing estrogenic therapy. The second patient with ovarian agenesis (*M.H.*) had an excretion of 85 R.U. by the same technic. The urine of the third patient (*B.W.*), assayed at the laboratory of the Massachusetts General Hospital by the technic of Klinefelter, Albright and Griswold, showed 78 M.U. per 24 hours.

Sexual Hair and 17-Ketosteroid Excretion. The presence of pubic and axillary hair at a time when the breasts, vagina and uterus remain undeveloped is strong evidence that the adrenals are producing androgens while the ovaries have failed to elaborate estrins. An increase of the excretion of 17-ketosteroids

above the pre-adolescent level offers the same evidence. According to Talbot et al. (26) the 17-ketosteroid output per day is under 1 mg. for a child of either sex under 6 years of age; at 8 years the range is from 0 to 2 mg. with an average of 1 mg.; at 12 years from 2 to 5 mg. with an average of 3.4 mg. per day. A normal child should excrete at least 1 mg. after it has passed its 12th birthday. In generalized hypopituitarism in which there is a deficiency of both gonadotropic and adrenotropic hormones pubic hair is lacking while in sexual infantilism dependent upon ovarian deficiency alone and in the rare cases of specific deficiency of gonadotropic hormone sexual hair is present. One must remember, however, that there are wide variations in both the pattern and the time of normal adolescence. Although breasts usually develop at the same time or shortly before the appearance of pubic hair, in some girls the sexual hair appears long before the development of the breasts. One must be careful not to mistake a case of delayed adolescence with pubic hair appearing earlier than breast development for one of true primary ovarian deficiency.

Skeletal Development. As shown in figures 1 and 2, patients with sexual infantilism show marked differences in their heights and skeletal configurations. It is difficult to explain these differences although it is obvious that they depend upon two factors: (1) the capacity for growth and (2) the length of time during which skeletal growth continues before being terminated by fusion of the epiphyses. It is apparent that of itself the skeletal type of the individual does not permit a diagnosis of the cause of the ovarian deficiency. Patients with *ovarian agenesis* are almost always markedly stunted in growth although an exception was found in the patient of Robert Meyer (6). The lower skeletal segment, measured from the symphysis pubis, is at times relatively decreased in relation to the total height as in a case we reported (fig. 1A). Possibly due to the influence of androgen, the epiphysial development is only slightly retarded and epiphysial fusions may occur at nearly the normal age as in our patient whose epiphyses were nearly all fused at the age of 19 years. We believe that the stunted growth associated with ovarian agenesis is due to genetic rather than endocrine influences. In hypopituitary dwarfs (fig. 2A) the upper and lower skeletal segments are usually in normal proportions for the patient's *age* and accordingly, in the young patient the lower segment may be slightly longer than the average for the patient's *height*. The epiphysial development is more markedly retarded than in primary ovarian agenesis and epiphysial fusions may not occur at all or only late in life. In a female dwarf showing no development of sexual characteristics one should suspect ovarian agenesis if pubic hair is present; hypopituitarism if it is absent. When the ovaries are removed or destroyed before the age of puberty the eunuchoid skeletal type results; the patient is tall, slender with relatively long legs and arms (fig. 1B). It seems probable that some cases of arrested ovarian development may result in this type. Our patient with "specific deficiency of the pituitary gonadotropins" (fig. 2C) showed the same eunuchoid proportions. The syndrome of sexual infantilism, obesity and diabetes insipidus described by Froehlich and due to extra-sellar lesions may be accompanied by some stunting of growth and at the same time eunuchoid skeletal proportions, as shown in our patient (fig. 2B).

Studies of Other Pituitary Functions. The detection of disturbances of the various functions of the pituitary still remains a matter of considerable difficulty in spite of the illuminating studies of Albright and his co-workers (11, 12). Fraser and Albright (11, 12) have introduced the term "panhypopituitarism" to indicate certain cases in which there appears to be a deficiency of all the numerous pituitary functions, viz. (1) growth; (2) gonadotropic stimulation; (3) thyrotropic stimulation; (4) adrenotropic stimulation; (5) the regulation of carbohydrate metabolism (which in turn may be mediated through the adrenal). According to these workers, such patients are dwarfed and show delayed epiphysial development. They remain sexually undeveloped. The B.M.R. is usually within normal limits but sometimes decreased if there is an associated thyroid deficiency. The excretion of 17-ketosteroids is said to be reduced below the level of 0.5 mg. per day. The disturbance of carbo-

hydrate regulation is demonstrated by the failure to respond to a hypoglycemia induced by the intravenous injection of insulin. In our own experience with patients between the ages of 12 and 18 years, even complete studies such as these have frequently left us in doubt as to whether stunted, sexually infantile patients are suffering from a true pituitary defect or are merely instances of a delay in the activation of the pituitary mechanism which initiates adolescence and its accompanying spurt of growth. Neither the assay of 17-ketosteroids nor the "response to hypoglycemia" have given as clear-cut a differentiation between the two conditions as we might desire.

We have pointed out that sexual infantilism may result from deficiency of the pituitary gonadotropins in cases where some or all of the other functions of the pituitary remain undisturbed. One of our patients (*B.S.*) was a dwarf but did not show the failure of response to hypoglycemia or the low excretion of 17-ketosteroids. Another example is the so-called Froehich's syndrome which is usually attributable to a lesion such as a suprasellar cyst in the hypothalamic region rather than a destruction or defect of the pituitary gland itself. The other example is the "selective deficiency of pituitary gonadotropin" which we and Albright have described. Such a condition may be strongly suspected in a patient well beyond the normal age of puberty and not stunted in growth if there is a low titer of urinary gonadotropin, complete failure of development of the breasts, vagina and uterus but a normal growth of sexual hair.

Exploratory Laparotomy and Peritoneoscopy. The various studies described above aid considerably in suggesting the cause of sexual infantilism, although we have attempted to point out some of the difficulties in the interpretation of the findings. We believe that a certain diagnosis of the condition of the ovaries can be established only by biopsy. In the present state of our knowledge it seems important to determine the exact ovarian pathology and correlate the laboratory findings with this rather than to base the diagnosis upon laboratory methods which have not been firmly established. An exploratory laparotomy to permit a direct inspection of the ovaries and excision of a small piece of tissue for biopsy is comparatively simple. In lieu of this, peritoneoscopic examination is fairly satisfactory.

TREATMENT

Primary Ovarian Deficiency. The rational treatment of primary ovarian deficiency consists in substitution therapy with estrogenic substances such as stilbestrol, estradiol esters or possibly the "naturally occurring estrogens" prepared from pregnant mares' urine. If stilbestrol is well tolerated the results with it are as satisfactory as with the other substances. In fact, Woodruff and TeLinde (27) believe that it causes greater growth of the breasts and the uterus in children than the natural estrogens if doses producing a comparable vaginal response are used. The oral administration and low cost of treatment are decided advantages. It has been our plan to give continuous treatment in doses of 1 mg. or 0.5 mg. daily until the uterus reaches the usual size attained in late adolescence unless endometrial bleeding occurs earlier. This may require a year or more. During treatment the vaginal epithelium undergoes rapid conversion to the adult type. The vaginal secretions increase greatly and the smear shows full estrin effect. With stilbestrol treatment a dark pigmentation of the areolae appears at first, which later fades out somewhat. The breasts develop fairly rapidly to a certain point. At times the breasts become almost cylindrical rather than of the normal conical shape. Whether the breast development is entirely normal or whether there is excessive hyperplasia of the ducts in relation to the alveolar structure, is open to question (28, 29). When the uterus attains nearly the normal adult size, there is danger that the continuous administration of estrins may bring about a hyperplasia of the endometrium with bleeding. It is therefore advisable at this point (or if bleeding occurs at an earlier stage of treatment) to institute cyclic interruptions of estrin therapy in order to permit endometrial degeneration and "artificial menstruation." In order to simulate physiological conditions as closely as possible, we have preceded the withdrawal of stilbestrol with the administration of a progestin for a period of 6 days. For this purpose, we have given anhydrohydroxy-

progesterone.[4] Although we have given daily doses of 10 mg. by mouth, TeLinde has found that 30 mg. a day are more effective in producing secretory changes in the endometrium. The treatment is arranged in monthly cycles, thus: (1) stilbestrol alone, 1 mg. daily for 15 days; (2) stilbestrol 1 mg. plus anhydrohydroxy-progesterone 10 mg. for 6 days; (3) no treatment for 7 days. Usually uterine bleeding occurs two days after withdrawal and lasts for several days.

Treatment of this sort results in sexual development which closely simulates that of the normal female adult. Treatment must be continued indefinitely. The development of the vagina reaches a sufficient size to make marital relations quite possible. Such a patient should be made to understand plainly that fertility is impossible. If marriage is entered into, it should be with this distinct understanding and the condition should be explained to both parties. Before treatment these patients have marked feelings of inferiority and are morose and discouraged. With the attainment of adult attributes they show marked psychic improvement, develop self-confidence and become more mature in their attitudes.

Sexual Infantilism Due to Pituitary Deficiency. The rational treatment of sexual infantilism due to pituitary deficiency would be the administration of the follicle-stimulating hormone or combinations of follicle-stimulating and luteinizing hormones. Unfortunately, none of the preparations available is entirely satisfactory. The widely used chorionic gonadotropins prepared from pregnancy urine contain principally luteinizing hormones and have not given satisfactory results in females. The serum prepared from pregnant mares is said to contain both follicle-stimulating and luteinizing hormones. Although they should be more suitable for the treatment of such cases, they have the serious drawback of containing foreign protein. The continuous or constantly repeated use of such a substance at times results in the sensitization of the patient to the foreign protein so that treatment has to be stopped (30). A product consisting of a combination of pregnancy-urine hormone and pituitary extract has been given therapeutic trial. We have had no experience with it so far. Hormones prepared from the urine of menopausal women should theoretically give the desired follicle-stimulating effect and yet be free from excessive protein. Such a preparation has not yet been made available for therapeutic purposes to our knowledge. Geist et al. (31) have studied the effect of various gonadotropins on human ovaries in a large series of cases and find that none induced maturation of the follicles. With hypophysial gonadotropins they found enlargement of cystic follicles, proliferation of granulosa cells, luteinization of the theca interna, whereas they observed only perifollicular congestion and hemorrhage with chorionic gonadotropins. No synergistic effect of combinations of hypophysial with chorionic gonadotropins was noticed. Recently Davis and Hellbaum (32) have reviewed the therapeutic use of gonadotropic preparations in females.

Although the use of the various gonadotropic preparations is worth a trial in the cases of pituitary deficiency, the therapeutic results are usually unsatisfactory. When failure is encountered, substitution therapy with estrins is justifiable according to the plan outlined above. It must be realized, however, that such substitution will probably lead eventully to atrophy of the patient's ovaries and will remove the possibility of fertility. In cases of pituitary dwarfism in which the absence of sexual hair indicates that there is also a deficiency of adrenal androgens, we have combined androgenic and estrogenic therapy. Estrins alone cause no marked retention of nitrogen, no acceleration of growth, and no development of sexual hair. All of these results are accomplished by the addition of androgens. We have given 10 to 25 mg. of methyl testosterone[4] daily in addition to 1 mg. stilbestrol. Such treatment has caused an adolescent spurt of growth similar to that brought about by androgens in sexually infantile, dwarfed boys. Usually no evidence of virilization is apparent. With the larger doses, however, we have encountered in one or two cases slight deepening of the voice and slight enlargement of the clitoris necessitating reduction of the dose. Pubic hair develops normally when androgen is given

[4] The methyl testosterone (Metandren) and the anhydrohydroxy-progesterone (Lutocylol) used were kindly supplied by the Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

but disappears again if it is discontinued. That the results are sometimes very satisfactory is shown in the case of a patient who has been under treatment since January, 1941 (a period of $3\frac{1}{2}$ years). Before beginning combined estrogenic-androgenic therapy she was 22 years old, 56.4 inches tall, pudgy, without any development of breasts or sexual hair, and with labia, vagina and uterus of infantile size. Not only were her features childish, but her emotional interests were immature. She was sensitive, worried and discouraged about her condition. After 3 years of treatment she appears to be a normal and very attractive young woman, 58.8 inches tall, with mature features, feminine body contours, well developed breasts and pubic hair. The vagina and uterus are of adult size. She was recently married and able to partake of normal marital relations. She is happy and vivacious in spite of the fact that she realizes that she is sterile. This case will be reported in detail in a later paper.

SUMMARY

Sexual infantilism in females may result either from primary ovarian deficiency or from a failure of the pituitary to produce the gonadotropic hormones. In the first group are cases due to ovarian agenesis and to destruction or removal of the ovaries before puberty. In the second group are cases of pituitary dwarfism, of Froehlich's syndrome and of specific deficiency of pituitary gonadotropin. The clinical findings in these various conditions are illustrated and discussed. The value of diagnostic studies including the assay of F.S.H. in the urine and the determination of the urinary excretion of the 17-ketosteroids is considered. The importance of exploratory laparotomy or peritoneoscopy is stressed. Indications for various types of treatment are discussed. Plans of treatment with estrogen and progestin and with androgen in proper cases are described. A case is reported of a patient with pituitary infantilism who was successfully treated.

Dr. Richard W. TeLinde, professor of Gynecology at the Johns Hopkins University, performed the gynecological operations and made the gynecological and pathological examinations. Without his help and advice this work could not have been carried out.

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Perlingual Absorption of Progesterone and Anhydrohydroxyprogesterone[1,2]

II. Clinical Evaluation

ROBERT B. GREENBLATT, M.D.[3]

Augusta, Georgia

PROOF for the sublingual absorption of such steroids as estradiol, desoxycorticosterone, methyl testosterone or anhydrohydroxyprogesterone has not been convincing inasmuch as part of these preparations may be swallowed. Progesterone, however, is inactivated by ingestion. If it can be shown that progesterone is effective when administered sublingually it would afford proof that the other steroids are also absorbed by way of the oral mucosa.

Observations were made on 68 patients following 141 courses of therapy. Sublingually anhydrohydroxyprogesterone was used in 94 courses and progesterone in 47 courses. Priming with or the concurrent administration of estrogens with progestins was employed in 17 instances. Endometrial biopsies were taken when feasible prior to and after administration of a course of therapy.

The criteria for effective absorption of each preparation were:

1) the induction of "progesterone withdrawal bleeding" in amenorrheic patients;

2) the effective arrest of menorrhagia 6 to 10 days after withdrawal of the drug, i.e., "arrest of bleeding according to plan";

3) the effectual absorption of these preparations was further surmised from the beneficial effects noted in patients with mastodynia, premenstrual tension, and certain patients with dysmenorrhea, as well as its varying effect on sexual libido. Occasionally in lieu of the withdrawal bleeding that was expected following sublingual medication, the patients experienced molimina, breast turgidity or uterine cramps.

The Induction of "Progesterone Withdrawal Bleeding" in Amenorrheic Patients. The patients that comprise this series were classified as follows.

A. Amenorrhea. Included in this group were cases of primary, secondary and functional amenorrhea, as well as those patients who exhibited the amenorrhea-menorrhagia syndrome of metropathia hemorrhagica and were observed during the amenorrheic phase.

B. Patients who were treated during the amenorrheic phase, after the correction of a prolonged bout of menorrhagia.

It appeared that when menorrhagia was successfully arrested by hormonal therapy, cyclical bleeding occasionally ensued. However, an amenorrheic phase usually followed. Experience has taught that if recurrence of further bouts of menorrhagia is to be prevented uterine bleeding could be and should be induced by progesterone withdrawal therapy. Courses of therapy were administered for several months with subsequent regulation of menses in many instances. Frequently, however, a return to the previous status of menorrhagia occurred which necessitated continuation of treatment.

Progesterone linguets were administered to 21 amenorrheic women and anhydrohydroxyprogesterone to 25. Many received courses of one or the other of the sublingual progestins

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[2] Aided by a Grant-in-aid of Research to the University of Georgia School of Medicine by the Ciba Pharmaceutical Products, Inc.

[3] This work was completed before the author's entrance into the Service. Present address is Robert B. Greenblatt, Surgeon (R), U. S. Public Health Service, Medical Center, Hot Springs, Arkansas.

on different occasions so that comparative studies could be made in the same patient. In all, 29 courses of progesterone and 50 courses of anhydrohydroxyprogesterone linguets were administered. Priming with estrogen or its concurrent use with the progestin medication was employed in 4 and 13 instances respectively. Dosage varied from 45 to 250 mg. over a period of 3 to 10 days. The average dosage, however, was 100 to 150 mg. Table 1 summarizes the pertinent data.

Interpretation. Withdrawal bleeding occurred with progesterone linguets in 55 per cent (16 of 29 instances) and in 74 per cent (37 of 50 instances) with anhydrohydroxyprogesterone when applied sublingually. The doubtful results were those in which bleeding occurred before medication was completed or when too long an interval (i.e., more than 10 days) elapsed before the uterine bleeding occurred. Several failures with perlingual progestin therapy may be attributed to the smallness of the dosages, i.e., 45 to 60 mg. The frequency with which withdrawal bleeding occurred after either progesterone or anhydrohydroxyprogesterone linguet therapy provided unquestionable proof of the absorbability and utilization of these preparations. Furthermore, though the onset of bleeding before medication was completed is herein included as a doubtful result, it may yet prove to be indicative of progesterone utilization.

TABLE 1. PROGESTIN LINGUET MEDICATION IN AMENORRHEA

Medications	No. of Patients	Courses	Effective	Questionable	Failure
Progesterone linguets		25	15	6	3
Primed with estrogens		4	1	1	2
	21	29	16	7	5
Anhydrohydroxyprogesterone linguets		37	30	3	3
Primed with estrogens		13	7	1	5
	25	50	37	4	8

The Effective Arrest of Menorrhagia

Twelve patients with menometrorrhagia were treated with a course of anhydroxyprogesterone. The dosages varied from 75 to 200 mg. over a period of 3 to 5 days. In 8 instances bleeding was arrested according to plan (table 2). In the remaining four, adjunctive hormonal measures were necessary in two instances successfully to arrest the bleeding which did not seem to be slowing down by the ninth day after withdrawal of therapy, and in two instances bleeding stopped during medication and withdrawal bleeding did not ensue. Three patients with menometrorrhagia received courses of progesterone linguets. In one patient bleeding was arrested according to plan; in another, bleeding stopped on the last day of medication and was not followed by withdrawal bleeding; and in the third, bleeding abated during therapy and then withdrawal bleeding followed and persisted for 3 weeks.

Definition of "Arrest of Bleeding According to Plan"

When progestins are administered for 3 to 5 successive days, either parenterally in doses of 5 to 10 mg. daily or orally in the form of anhydrohydroxyprogesterone in doses of 100–150 mg., the following may be expected: the bleeding may cease, slow down or continue unabated during therapy. About 2 or 3 days after withdrawal of medication the bleeding suddenly may increase, continue for three or four days and then may stop abruptly or taper down and halt slowly. In all, uterine bleeding continues for 6 to 10 days after cessation of therapy. Unless this phenomenon, the "arrest of bleeding according to plan" is appreciated, the clinician may conclude that progesterone therapy increases bleeding and accordingly is contraindicated (1).

Observations in Miscellaneous Conditions

A. Mastodynia. Six patients who experienced cyclic or constant breast pain, so marked as to seek medical aid, are included in this group. For the alleviation of cyclomastopathy, two patients received progesterone and one re-

TABLE 2. PROGESTIN LINGUET MEDICATION IN MENOMETRORRHAGIA

Linguet	No. of Patients	Effective	Questionable	Failure
Anhydrohydroxyprogesterone	12	8	2	2
Progesterone	3	1	1	1

ceived anhydrohydroxyprogesterone linguets in doses of 5 to 10 mg. daily during the last half of the cycle over a period of several months. The results were satisfactory. One young woman with puberal breast hypertrophy experienced remarkable relief of annoying breast pain along with detumescence of the breast turgidity, following administration of 1 or 2 linguets daily of anhydrohydroxyprogesterone over a period of 10 weeks. Improvement was not noticeable until the fifth or sixth week of therapy. Two women suffering with mazoplasia and mastodynia received adequate relief from courses of anhydrohydroxyprogesterone sublingually.

B. Nervous Tension States. Major menstrual molimina were marked in 4 patients. Partial or complete relief of premenstrual tension was noted following cyclic use of anhydrohydroxyprogesterone in doses of 1 linguet (5.0 mg.) daily for 10 or 15 days prior to onset of the expected menses. A fifth patient noted an exacerbation of the molimina following such therapy. One young woman who found herself in a constant state of nervous tension claimed a sedative effect from the daily use of 5 mg. of progesterone sublingually.

C. Dysmenorrhea. Thirteen courses of anhydrohydroxyprogesterone linguets were administered to 7 patients and 3 courses of progesterone linguets to 3 patients with dysmenorrhea. The dosage was 1 or 2 linguets (5.0 mg.) a day for 7 to 10 days prior to onset of menses. Table 3 gives the result of therapy. Two patients who obtained relief with this form of medication during the first trial failed to obtain relief following a second trial. This observation is difficult to evaluate. The psychogenic role in certain therapeutic measures in dysmenorrhea is also a matter for consideration. It is worth noting that one patient who regularly menstruated without pain experienced dysmenorrhea following the administration of anhydrohydroxyprogesterone linguets. Such experiences have been encountered more frequently when using progestins parenterally than orally (2).

D. Sexual Libido. Of the patients receiving anhydrohydroxyprogesterone linguets, several volunteered information concerning the effect of medication on sexual libido. Two experienced a definite increase; four noted a marked decrease; others stated there was no variation in their libido. Two patients receiving progesterone linguets claimed that sexual libido increased. In a previous study it was noted that libido decreased after large doses of progesterone parenterally or after pellet implantation (3). Small doses of progestins with or without estrogens frequently increased libido (4).

TABLE 3. PROGESTIN LINGUET MEDICATION IN MISCELLANEOUS CONDITIONS

DYSMENORRHEA

Linguet Medication	No. of Patients	Courses	Relieved	Partial Relief	Failure
Anhydrohydroxyprogesterone	7	13	7	3	3
Progesterone	3	3	1	0	2

MASTODYNIA

	No. of Patients	Improved	Failure
Anhydrohydroxyprogesterone	4	4	0
Progesterone	2	2	0

LIBIDO

	No. of Patients	Increased	Decreased
Anhydrohydroxyprogesterone	6	2	4
Progesterone	2	2	0

NERVOUS TENSION STATE

	No. of Patients	Improved	Exacerbated
Anhydrohydroxyprogesterone	5	4	1
Progesterone	1	1	0

Untoward Effects. In two instances medication was discontinued because of nausea. This could not be interpreted as a toxic reaction but rather as one due to the annoying presence of foreign particles in the buccal cavity. One patient complained of soreness of the buccal mucosa following linguet application. Occasionally some patients complained that breast

turgidity resulted from this form of medication and one patient experienced painful menses subsequent to each course of therapy. Molimina and uterine cramps were frequently observed in those instances when withdrawal bleeding failed to occur.

Comparative Values. Allen (5) claimed that the ratio of effectiveness of anhydrohydroxyprogesterone perorally and progesterone parenterally was approximately 10:1. Joel (6) found it required anhydrohydroxyprogesterone perorally in 5 to 8 times the dose that was required if progesterone were injected. On the other hand, by sublingual administration the same effect could be obtained with about 3.5 times the injected dose. In the series reported herewith, information was culled from certain cases (*a* to *e*) which gave an insight of comparative values of the various progestins when used on different occasions in the same patient to induce withdrawal bleeding.

In (*a*) 45 mg. of progesterone sublingually failed to induce withdrawal bleeding while 75 mg. of anhydrohydroxyprogesterone sublingually was successful. Thirty mg. of progesterone parenterally induced withdrawal bleeding on several occasions.

In (*b*) 50 mg. progesterone sublingually failed but on a subsequent trial 75 mg. succeeded in producing withdrawal bleeding. In this same patient, 100 mg. anhydrohydroxyprogesterone sublingually and 150 mg. anhydrohydroxyprogesterone perorally also were successful.

In (*c*) 150 mg. of anhydrohydroxyprogesterone perorally failed to induce withdrawal bleeding. On subsequent trials withdrawal bleeding was accomplished by the sublingual use of 105 mg. of progesterone and also by a similar dose of anhydrohydroxyprogesterone sublingually.

In (*d*) 60 mg. of anhydrohydroxyprogesterone sublingually failed to induce withdrawal bleeding while at a later data 120 mg. was employed successfully.

In (*e*) 15 mg. of progesterone parenterally, 90 mg. of anhydrohydroxyprogesterone sublingually and 110 mg. of progesterone sublingually each succeeded in bringing about withdrawal bleeding.

In attempting to analyze the data collected in this study it appeared that the minimal dosage required to produce withdrawal bleeding with progesterone parenterally was 15 mg. while the average dose varied from 20 to 30 mg. The minimal dose of progesterone or anhydrohydroxyprogesterone sublingually was about 75 mg., while the average dosage for the former varied from 125 to 150 mg. and for the latter, from 100 to 125 mg. From an analysis of the literature it would appear that the minimal dose for anhydrohydroxyprogesterone by ingestion was 125 mg., while the average varied from 150 to 200 mg. In this study, however, the experiences gained with anhydrohydroxyprogesterone by ingestion in producing withdrawal bleeding were too limited to warrant conclusions (table 4).

TABLE 4. DOSAGES REQUIRED TO PRODUCE WITHDRAWAL BLEEDING FOLLOWING ADMINISTRATION OF VARIOUS PROGESTINS

Dosage	Progesterone Parenterally	Progesterone Sublingually	Anhydrohydroxy-progesterone Stomachally	Anhydrohydroxy-progesterone Sublingually
Minimal	15 mg.	75 mg.	125 mg. (?)	75 mg.
Average	20–30 mg.	125–150 mg.	150–200 mg. (?)	100–125 mg.

It may be assumed that the relative ratio of effectiveness between progesterone parenterally and progesterone or anhydrohydroxyprogesterone sublingually is about 5–6:1. Judging by the effect on withdrawal bleeding (successful in 74 per cent in comparison with 55 per cent) anhydrohydroxyprogesterone sublingually appeared more effective than progesterone sublingually. Both preparations sublingually were at least as effective as anhydrohydroxyprogesterone by ingestion. There can be no doubt that progesterone sublingually is utilized; that it is not as effective as anhydrohydroxyprogesterone sublingually may be due to the fact that part of the material is inadvertently swallowed. The dosage required for therapeutic effectiveness is much below that capable of producing progestinal changes in the

endometrium. Endometrial studies were performed following sublingual application of progesterone or anhydrohydroxyprogesterone in twenty instances. In general, with dosages of 150 mg. or less, the endometrium persisted in the estrogenic phase while with the dosages from 150 to 250 mg. poor to moderately good secretory changes were obtained occasionally (7).

The results in the clinical evaluation of progesterone sublingually match the recent findings of Corner (8) in the experimental animal. The minimal effective dose of progesterone sublingually required to protect the adult castrate Rhesus monkey from estrogen deprivation bleeding was 4 or 5 times the intramuscular dose. Furthermore, "withdrawal progesterone bleeding" occurred 2 to 8 days afterwards in 5 of the 12 instances in which estrogen deprivation bleeding was inhibited.

SUMMARY

Observations were made on 68 patients following the sublingual administration (linguets) of 47 courses of progesterone and 94 of anhydrohydroxyprogesterone.

The linguets were made in the form of hard pressed, slowly soluble tablets which contained either 5 mg. of anhydrohydroxyprogesterone or 2.5 or 5 mg. of progesterone.

The main criterion for effective absorption of each preparation was the induction of "progesterone withdrawal bleeding" in amenorrheic patients. Four to ten linguets were given at varying intervals each day for 3 to 10 days. In the instances in which progesterone and anhydrohydroxyprogesterone were used, withdrawal bleeding occurred in 55 and 74 per cent of the cases respectively.

The effectual absorption of these preparations was further surmised from the clinical results obtained in menorrhagia, mastodynia, premenstrual tension and dysmenorrhea.

It may be assumed that the relative ratio of effectiveness between progesterone sublingually and parenterally is about 5 or 6:1 and these values are in satisfactory agreement with those obtained by Corner in the experimental animal (4 or 5:1).

Both progesterone and anhydrohydroxyprogesterone sublingually were at least as effective as anhydrohydroxyprogesterone by ingestion. There can be no doubt that progesterone sublingually is absorbed and utilized but due to the fact that part of the material is inadvertently swallowed and accordingly inactivated, it is slightly less effective than anhydrohydroxyprogesterone.

This clinical study, though limited in scope, permits the conclusion that steroids are effectively absorbed sublingually and that the perlingual administration of steroid hormones is a convenient method.

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An Evaluation of a Method for Measuring and a Survey of the Urinary Excretion of Weakly Phenolic Ketone, "Estrone", in Endocrine Disorders[1]

EDWARD C. REIFENSTEIN, JR., M.D., AND
ELEANOR F. DEMPSEY, A.M.

From the Department of Medicine of the Harvard Medical School and from the Medical Service of the Massachusetts General Hospital, Boston, Massachusetts

THREE ESTROGENIC SUBSTANCES have been identified in human urine: alpha estradiol, estrone and estriol (1). The excretion of these substances as a group and separately by normal individuals and by persons with endocrine disorders has been partially quantified by biological assays (2). Because of the recognized limitations of biological methods, efforts have been made to develop chemical procedures for assaying the estrogens in human urine (3).

These chemical methods until recently have been applicable only to the urine of pregnant women. However, in 1940, Talbot, Wolfe, MacLachlan, Karush and Butler (4) devised a chemical procedure which they reported had sufficient sensitivity to measure the excretion of weakly phenolic ketones in normal women. Since estrone is the only weakly phenolic ketone known to be present in extracts of hydrolyzed human urine, they suggested this test as a means of determining the human excretion of estrone. The assay involves the formation of a colored compound by coupling the phenolic hydroxyl group of estrone with diazotized dianisidine.

The present paper is concerned with some experimental studies of this chemical method and with a survey, by means of this test, of the urinary excretion of weakly phenolic ketone (estrone) in endocrine disorders.

[1] The expenses of this investigation were defrayed by a grant from the Josiah Macy, Jr., Foundation of New York.

ANALYTICAL PROCEDURE

Although in general the procedure was followed as outlined by Talbot, et al. (4), it was found necessary to modify or augment the original instructions at certain points. The unaltered procedure, where still followed, will be given in quotation marks.

REAGENTS

The reagents specified in the original description of the method were employed in the initial experiments. After a time difficulties were encountered which ultimately were traced to impurities in the solvents.

Ethyl Ether. Several types of impurities were found in varying quantities in several commercial grades of ethyl ether. 1) *Substances giving phenol reactions.* Of twenty-eight 150-cc.[2] samples tested, 15 gave as much color with the phenol reagent as was produced by 10 to 100 micrograms of estrone, 6 gave as much color as 100 to 150 micrograms of estrone, and only 7 gave no appreciable color. These substances were increased by storage. 2) *Substances inhibiting phenol reactions.* There was at times in 150 cc. enough of these impurities to reduce the color given by a standard solution of estrone by nearly 60 micrograms. 3) *Substances with color.* Some samples when evaporated to dryness left dark-brown, dirty residues which

[2] This is the quantity added to Step III of the analytical procedure after the treatment with Girard's Reagent T (vide infra, Ether Blanks, and Step III).

partially obscured the color produced by the phenol reagent with estrone.

The nature of these impurities was not determined. They could be completely removed by treatment with Girard's Reagent T; and they could be reduced to a considerable degree by treatment with the ferrous sulfate cleaning procedure (vide infra).

Ether Cleaning Procedure. Prepare a solution of 1 teaspoonful of powdered ferrous sulfate C. P. in 100 cc. 1 N sulfuric acid. Wash about 2 liters of ethyl ether in a 2-liter separatory funnel with four 25 cc. portions of this ferrous sulfate-sulfuric acid solution. Then wash the ether with four 25 cc. portions of 1 N NaOH. Finally wash the ether with 25 cc. portions of distilled water until the washings are neutral to litmus. All washings are discarded. All the ether is then redistilled. The ether should be used the same day it is cleaned, as interfering substances may develop if it is stored.

Toluene. Two 150-cc. samples contained impurities which gave as much color with the phenol reagent as was produced by 21 and 18 micrograms of estrone respectively. These substances could be removed completely by treatment with Girard's Reagent T.

Carbon Tetrachloride. Carbon tetrachloride C. P. was used for the initial extraction in Step I (vide infra) instead of ethyl ether as described in the original method. Four 150-cc. samples contained impurities which gave as much color with the phenol reagent as was produced by 208, 207, 145 and 106 micrograms of estrone respectively. These substances could be removed completely by treatment with Girard's Reagent T.

Ether Blanks. Since Girard's Reagent T, which is employed in Step III of the analytical procedure, was found to remove all of the impurities introduced through the solvents, the impurities added before Step III were thereafter ignored. Subsequent to the employment of Girard's Reagent T in Step III, only 150 cc. of ethyl ether is added to the specimen being analyzed. Therefore, it was decided to use only cleaned ether after the treatment with Girard's Reagent T, and in addition to run a blank on every batch of this ether. The assay values were corrected for the value of the "ether blank." To test the efficacy of this procedure in eliminating impurities, 150 cc. of carbon tetrachloride was placed in each of six flasks and evaporated to dryness; the entire analytical procedure beginning with Step I (vide infra) was then carried out for these flasks with the use of cleaned ether and ether blanks. Complete removal of all impurities was demonstrated.

Other Reagents. The list in Talbot's paper (4) should be consulted.

COLLECTION, HYDROLYSIS AND EXTRACTION OF URINES

The apparatus is all-glass throughout, and is always chemically-cleaned, except for the continuous extraction apparatus. The 24-hour urine sample is collected without preservative; it is kept cool during the collection period and hydrolyzed and extracted within 6 hours after the collection is completed.

Fifteen volumes per cent of commercial HCl is added; the acidified urine is placed in a steam hydrolyzer of 1500 cc. capacity, and hydrolyzed for exactly 10 minutes after it begins to boil. Then the hot urine is run at once into a Hershberg-Wolfe continuous-extraction apparatus (5) and extracted with about 500 cc. of carbon tetrachloride for 45 minutes. The carbon tetrachloride extract is evaporated to dryness and the residue transferred with 190 cc. of cleaned ethyl ether to a 250 cc. separatory funnel. "The final volume is adjusted to 190 cc., so that in the subsequent purification, the amount of air in the separatory funnel is not excessive."

STEP I. Treatment with Weak Alkali

"The strongly acidic and phenolic substances are extracted from the ether by washing alternately with three 25 cc. portions of 0.1 N NaOH and two 25 cc. portions of 0.1 N NaOH-sodium hydrosulfite reagent. In the hydrosulfite-alkali extractions, the separatory funnel is tightly stoppered and shaken thoroughly for at least 3 minutes (or until the mixture is yellow in color). The ether is then washed twice with 25 cc. portions of 0.5 N HCl and three times with 25 cc. portions of water. All the washings are discarded." The remaining ether solution is transferred quantitatively to a distilling flask with 3 or 4 portions (totalling 25 cc.) of cleaned ether and then evaporated to dryness.

STEP II. Treatment with Strong Alkali

The residue from Step I is transferred quantitatively to a clean 250 cc. separatory funnel with four

25 cc. portions of toluene. "The weakly phenolic substances are extracted from the toluene with four 25 cc. washings of 1 N NaOH. The combined 1 N NaOH extracts are made acid to Congo red paper (blue) with about 10 cc. of C. P. HCl and then adjusted to a pH that is acid to litmus and alkaline to Congo red by the addition of 20 per cent Na_2CO_3 solution." It was found that this pH adjustment must be made very carefully. This solution is cooled and transferred to a clean separatory funnel with five 40 cc. portions of cleaned ethyl ether with which it is then extracted. "The combined ether extracts are washed once with 25 cc. of 0.1 N NaOH, once with 25 cc. of 0.1 N NaOH–$Na_2S_2O_4$ reagent, shaken at least 3 minutes, washed once with 25 cc. of 0.1 N NaOH, twice with 25 cc. of 0.5 N HCl, and three times with 25 cc. portions of water." This purified ether extract is transferred quantitatively to a distilling flask with 3 or 4 portions (totalling 25 cc.) of cleaned ether and then evaporated to dryness.

STEP III. Treatment with Girard's Reagent T.

To the residue from Step II in the same flask are added 200 mg. of Girard's Reagent T and 0.5 cc. of glacial acetic acid; the mouth of the flask is covered with tinfoil held on with a rubber band, and the flask is heated on a rapidly boiling hot water bath for exactly 10 minutes. The flask is thoroughly chilled in an ice bath and then transferred with 40 cc. of ice-cold distilled water to a separatory funnel. Three cc. of 10 per cent aqueous NaOH solution is added. The mixture is then extracted as rapidly as possible four times with 40 cc. of cleaned ethyl ether. "These ether extracts (non-ketonic fraction) are discarded. The remaining aqueous mixture is delivered into a flask containing 1 cc. of concentrated H_2SO_4 in 60 cc. of distilled water. The acidified mixture is transferred to a clean separatory funnel." Twenty-five cc. of cleaned ethyl ether is added and the mixture is allowed to stand at room temperature for about 75 minutes. It is then extracted with the ether added and with four additional 25 cc. portions of cleaned ether. "The combined ether extract is washed once with 25 cc. of 0.1 N NaOH and three times with 25-cc. lots of water." After the final water is carefully drained from the ether it is transferred quantitatively to a distilling flask, with 3 or 4 portions (totalling 25 cc.) of cleaned ether and evaporated to dryness. The residue contains the weakly phenolic ketones. From the same batch, 150 cc. cleaned ethyl ether is evaporated to dryness in another flask as an "ether blank."

COLORIMETRIC ASSAY

For greater accuracy in delivering the small volumes of reagents required for the assay, a series of Krogh automatic syringe-type micropipettes are standardized, employing tuberculin or larger syringes fitted with 19-gauge, blunt, steel needles. In the studies reported, a Klett-Summerson photoelectric colorimeter adapted for micro-assay was used with Corning Signal Blue 556 and Light-Absorbing 398 filters. The micro-tubes in this instrument require 2 cc. of solution to be read accurately.

The assay is performed as follows.

A measured volume (usually 1 cc.) of absolute alcohol is added to the residue from Step III, and the residue, including any particles adhering to the sides of the flask, is dissolved by rotating and agitating the flask and then allowing it to stand for one hour. Of this absolute alcohol solution, 0.3 cc. is then carefully delivered into the bottom of a centrifuge tube. "Seventy-five hundredths cc. of 2 per cent sodium carbonate solution is then delivered into the tube. It is important to pipette these two solutions into the lowest part of the test-tube without wetting the side walls above the level of the meniscus." As rapidly as possible 0.1 cc. of the diazotized-dianisidine reagent is delivered and the mixture is stirred immediately for a few seconds with a glass rod. After 1 minute, 0.8 cc. of toluene is delivered into the tube. The tube is stoppered with a finger and shaken vigorously for a few seconds. A blank of the assay reagents and the "ether-blank" are prepared in the same way. The tubes are centrifuged at about 2500 R. P. M. for 5 minutes. "After centrifuging, the lower aqueous phase should be essentially colorless; the colored toluene layer should be clear and the color evenly distributed." With the special syringe-pipette 0.6 cc. aliquots of the clear supernatant toluene from the tubes containing the blank and unknown preparations are carefully transferred to the absorption tubes of the photoelectric colorimeter. The toluene in the tubes should be clear and free from water droplets or precipitate. To each of the tubes is added 1.4 cc. of toluene, so that the total volume in each tube is exactly 2 cc. (vide supra). By means of a filter with maximum transmission at 420 mμ, range 380 to 480 mμ, the galvanometer is adjusted to zero with a tube containing only toluene in place. This tube is then replaced by that containing the "assay-reagent-blank," and the resistance reading of the blank obtained; similarly the resistance reading of the "ether-blank" and of each unknown sample is obtained. In practice, duplicate "assay-reagent-blanks," duplicate samples of three unknowns, and duplicate "ether-blanks" are set up together (10 tubes). The number of micrograms of weakly phenolic ketone (estrone) is determined by subtracting the average resistance readings of the "assay-reagent-blanks" from the average resistance reading of each of the unknowns (including the "ether-blanks") and then correcting the unknown samples, if necessary, for any impurities that may have been measured in the "ether-blanks." The corrected resistance readings are then referred to a calibration curve in which resistance readings are plotted against known amounts of crystalline estrone (2.5 to 30.0 micrograms).

RECOVERY EXPERIMENTS

Because changes had been made in the analytical procedure as originally described, the recovery of estrone that could be obtained with the modified procedure was studied. As mentioned before, blanks run through the complete procedure were negative. Experiments were carried out to determine the recovery from standard solutions of estrone and from urine pools to which known amounts of estrone had been added.

Recovery from Standard Solutions. Standard solutions were prepared by dissolving weighed samples of crystalline estrone[3] in absolute alcohol. The recovery of these solutions was determined for each step in the analytical procedure separately, and similarly for the three steps in combination (table 1). It is to be noted that when the average percentage recoveries on the individual steps on different samples are summated [89 per cent (Step III) of 66.6 per cent (Step II) of 85 per cent (Step I)], an average recovery of 50.4 per cent is obtained, which is to be compared with the average recovery of 54.7 per cent obtained when the three steps are employed in sequence on the same sample. The small discrepancy is probably readily explained by the fact that the summation involves the addition of the slight losses involved in making three colorimetric assays (table 1).

Recovery from Urine Pools. In the recovery experiments on standard solutions, neither the influence of the process of extraction on the recovery of added estrone nor the possibility that unknown substances present in human urine might interfere with the recovery of added estrone could be studied. To examine these possibilities, several urine pools were studied. On the basis of a 55 per cent recovery (table 1), 500 cc. aliquots of three pools (A, B, and C) were found to contain less than 3 micrograms of weakly phenolic ketones per 0.3 cc. of alcoholic solution of the residue when it was taken up in 1 cc. Since the colorimetric assay was considered to be inaccurate for less than 3 micrograms per 0.3 cc., these pools were designated merely as <3 micrograms (table 2).

In performing recovery experiments with the urine pools, 500 cc. aliquots of the urine were hydrolyzed, 4 cc. of a standard alcoholic solution of estrone was added to each aliquot and then the aliquots were extracted with carbon tetrachloride. Thereafter, the complete analytical procedure was carried out. The recovery in Pools A, B, and C fell between 51 and 63 per cent (table 2). These values are to be compared with those obtained by Talbot and his associ-

TABLE 1. RECOVERY OF CRYSTALLINE ESTRONE FROM STANDARD SOLUTIONS IN VARIOUS STEPS[1] OF ANALYTICAL PROCEDURE

Analytical Procedure[1]	Estrone Added[2] γ	Estrone Recovered												
		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Average
		γ	%	γ	%	γ	%	γ	%	γ	%	γ	%	%
Step I	13.1	8.2	63	13.1	100	13.1	100	—	—	—	—	—	—	85
	13.5	—	—	—	—	—	—	12.6	93	10.5	78	10.4	77	
Step II	18.1	12.75	70	11.6	64	12.0	67	12.25	68	11.6	64	12.1	67	66.6
Step III	13.5	13.6	100	11.5	85	12.2	90	—	—	—	—	—	—	89
	18.1	—	—	—	—	—	—	13.75	76	16.3	90	16.75	93	
Summation: 89% (Step III) of 66.6% (Step II) of 85% (Step I)														50.4
Steps I, II and III	19.2	10.8	57	9.75	51	11.6	60	10.1	53	9.8	51	10.8	56	54.7
Colorimetric Assay	18.1	18.4	101.6	17.2	95.0	18.3	101.1	—	—	—	—	—	—	98.9
	19.2	—	—	—	—	—	—	18.9	98.4	18.9	98.4	—	—	

[1] For steps of analytical procedure see text.
[2] The added amount is considered to be the theoretical content.

[3] The authors are indebted to Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J., for the crystalline estrone, estradiol and estriol used in these experiments.

TABLE 2. RECOVERY OF CRYSTALLINE ESTRONE FROM HYDROLYZED URINE POOLS

(The results are expressed in micrograms per 0.3 cc. sample)

Pool No.[1]	Estrone in Pool γ	Estrone Added γ	Estrone Theoretical γ	Estrone Recovered						Average %
				Sample 1		Sample 2		Sample 3		
				γ	%	γ	%	γ	%	
A	<3	30.9	>30.9 to <33.9	16.6	>49 to <54	20.0	>59 to <65	16.4	>48 to <53	>60 to <73
	<3	10.9	>10.9 to <13.9	9.0	>65 to <83	9.5	>68 to <87	10.1	>73 to <93	
B	<3	12.0	>12.0 to <15.0	6.6	>44 to <55	6.2	>41 to <52	8.1	>54 to <67	>46 to <58
C	<3	12.0	>12.0 to <15.0	6.8	>47 to <57	7.0	>47 to <58	7.3	>49 to <61	>48 to <59
Average of Pools A, B, and C										>51 to <63
D	11.1[2]	9.8	20.9	11.5	55	11.2	54	12.0	57	55.6
	11.1[2]	9.8	20.9	9.7[3]	46[3]	12.2	58	11.2	54	
Average of all Pools										>55.3 to <59.3

[1] Pool A—males and females; Pool B—normal females; Pool C—normal males; Pool D—males and females.
[2] Three assays of this pool averaged 6.1 micrograms (4.5, 7.5, 6.2 micrograms) which was considered to be 55 per cent of an estrone content of 11.1 micrograms.
[3] Known loss; excluded from average.
<=less than; >=greater than

TABLE 3. URINARY EXCRETION OF WEAKLY PHENOLIC KETONES IN PERSONS WITH PHYSIOLOGIC GLANDULAR STATUS

Normal Females		
Subject Age	Day of Cycle	Weakly Phenolic Ketones[1] γ/24 hr.
R.G. 25	16th	38.3
	25th	37.6
	36th	27.1
E.C. 24	4th	11.6
	11th	13.0
	25th	29.0
	28th	17.5
D.B. 24	5th	<10[2]
	12th	14.7
	35th	24.2
E.M. 23	3rd	12.7
	10th	12.9
	25th	21.6
R.C. 23	13th	11.6
	20th	21.6
Average		20.2

[1] These values are thought to represent about 55 per cent of the amount actually present (see discussion in text).
[2] Value <10 averaged as 10 γ/24 hr.

Normal Males	
Subject Age	Weakly Phenolic Ketones[1] γ/24 hr.
L.K. 35	34.7
	38.0
E.R. 34	33.7
	27.7
D.B. 30	27.4
	42.2
M.G. 28	28.4
	40.6
E.B. 22	28.4
	27.7
Average	32.9

Pregnant Females	
Subject Age Month of Gestation	Weakly Phenolic Ketones[1] γ/24 hr.
A.F. 30 9th	675
	290
	510
R.S. 26 5th	175
	200
	440

ates (4) in estrone recovery experiments. In 6 experiments their recovery ranged from 57 to 79 per cent.

Another recovery experiment was performed on a urine pool (pool D) which contained an estimated 11.1 micrograms of weakly phenolic ketones prior to the addition of crystalline estrone (table 2). The recovery of weakly phenolic ketone plus added estrone averaged 55.6 per cent which agrees with the other recoveries.

TABLE 4. URINARY EXCRETION OF WEAKLY PHENOLIC KETONES IN PERSONS WITH PATHOLOGIC GLANDULAR STATUS

Diagnosis	Patient, Age Unit Number	Weakly Phenolic Ketones[1] γ/24 hr.
Females		
Menopause[2] and Addison's Disease	M.K., 48 #209947	<10 <10 <10
Addison's Disease	S.G., 46 #385496	25.7 30.4
Adrenogenital Syndrome	E.F., 26 #240632	49.8 47.8
Menopause[3] and Cushing's Syndrome	R.B., 53 #3397	24.3 29.3
Cushing's Syndrome	I.G., 11 #350260	11.8
Hypothalamic Amenorrhea (6)	H.T., 23 #317690	18.2 16.5
Acromegaly	A.W., 33 #395015	30.4 30.0
Acromegaly	C.S., 22 #347319	46.9
Females		
Menopause[2]	J.S., 36 #392243	37.6 35.3
Menopause[3]	L.M., 26 #373258	32.7 37.9
Menopause[3]	M.F., 42 #386427	27.6 34.1
Menopause[4]	B.D., 27 #384699	24.8 23.1
Menopause Average.....		31.6
Males		
Acromegaly	C.S., 44 #42654	20.8 36.6 20.6
Acromegaly	C.O., 20 #350940	29.7 46.2
Hyperthyroidism	P.C., 44 #369235	24.3 23.1
Gynecomastia and Small Testis Syndrome (7)	L.B., 30 #141135	38.0 48.2

[1] These values are thought to represent about 55% of the amount actually present (see text for discussion).
[2] Surgical.
[3] Physiological.
[4] Radium.

CLINICAL DATA

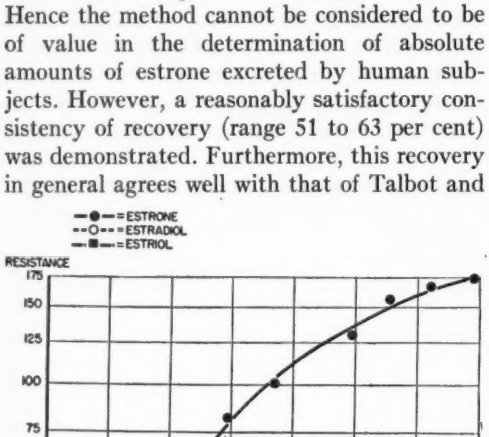
1) *Persons with Physiologic Glandular Status.* The micrograms of weakly phenolic ketone (estrone) excreted per 24 hours in the urine of women with normal menstrual cycles were studied (table 3). No correction was made in any of the clinical assays for the fact that the amount as determined apparently represents about 55 per cent (vide supra) of that actually present. Five normal women excreted between 10 and 39 micrograms per 24 hours; furthermore, there seemed to be somewhat less excreted during the first half of the cycle. However, daily determinations of the excretion of weakly phenolic ketone for a complete menstrual cycle were not made, hence it is not possible to state whether peaks were present. Two pregnant women were found to excrete between 200 and 440 micrograms per 24 hours during the 5th month and between 290 and 675 micrograms per 24 hours during the 9th month (table 3). Five normal males excreted between 27 and 42 micrograms per 24 hours (table 3).

2) *Persons with Pathologic Glandular Status.* A series of determinations was obtained on patients with endocrine disorders. These individuals excreted between 10 and 49.8 micrograms per 24 hours of weakly phenolic ketones (table 4).

DISCUSSION

Experimental Data. As pointed out by Talbot and his associates (4), the loss of estrone

(in the present experiment averaging 45 per cent) during the purification and isolation procedures is much greater than could be desired. Hence the method cannot be considered to be of value in the determination of absolute amounts of estrone excreted by human subjects. However, a reasonably satisfactory consistency of recovery (range 51 to 63 per cent) was demonstrated. Furthermore, this recovery in general agrees well with that of Talbot and



A comparison of the color produced by crystalline estrone, estradiol and estriol when their phenolic hydroxyl groups are coupled with diazotized dianisidine. The resistance readings were obtained on the galvanometer of a Klett-Summerson photoelectric colorimeter adapted for microassay and fitted with Corning Signal Blue 556 and Light-Absorbing 398 filters. For discussion, see text.

his associates (4). The method, therefore, might reasonably be applied to human excretion to obtain an indication rather than an absolute measure of the differences in states of health and disease.

The possibility of determining the phenolic content of the estradiol and the estriol fractions was explored by setting up standard curves against known amounts of crystalline estradiol and estriol (see accompanying figure). It was found that the color reaction was not suitable for measuring these two estrogens because in neither instance did it follow Beer's law. Furthermore, the amount of color produced by a given quantity of estrone did not correspond either to that given by the same quantity of estradiol or to that given by the same quantity of estriol; therefore this color reaction could not be used to measure the total content of these three estrogens in human urine.

Clinical Data. The excretion of weakly phenolic ketones by normal and pregnant women agrees with that obtained by Talbot and his associates (4) in such women. They reported that two normal women excreted between 25 and 60 micrograms per 24 hours, and that four pregnant women excreted between 325 and 600 micrograms per 24 hours during the 4th and 7th months of gestation. Furthermore, the excretion of weakly phenolic ketones by normal and pregnant women agrees fairly well with the estrone excretion of similar women as determined by the biological assays of Smith and Smith (8). These investigators reported that women excreted between 0 and 28 micrograms of estrone per 24 hours during a normal menstrual cycle, and between 166 and 890 micrograms of estrone per 24 hours during the 4th to the 9th month of a normal pregnancy. The excretion of weakly phenolic ketone, therefore, seems to correspond grossly to the level of physiological activity of the estrogen-producing glands in these normal individuals. This correlation warranted a survey of the excretion by patients with disorders of the endocrine glands.

It is apparent (table 4) that no significant differences could be demonstrated in the weakly phenolic ketone excretion in patients who had clinical evidence of disturbance in the glands thought to produce estrogens (gonads, adrenals). Women with menopause excreted as much or more than women with normal ovarian function. To be sure, some encouragement was derived from the fact that very small amounts were excreted by a woman with surgical menopause together with adrenal insufficiency (Addison's disease). However, another patient with Addison's disease and normal ovarian function excreted just as much as normal women. A significantly elevated excretion was not demonstrated in patients with increased function of the adrenal cortex (Cushing's syndrome, adreno-genital syndrome and acromegaly). The findings in males were equally disappointing. In fact, on the basis of this test alone, one could never differentiate a

male from a female, although frequently a distinct difference in the estrogen excretion can be demonstrated by bioassay (9, 10). Attention is called to the fact that the average weakly-phenolic ketone excretion of normal men was almost identical with that of menopausal women, whereas that of normal women was significantly lower. This suggests that the adrenal gland is the source of more urinary weakly phenolic ketones than the ovary, and that when the ovary is functioning the production by the adrenal gland is reduced.

Since the excretion of weakly phenolic ketone was found to be: 1) higher in menopausal than in normal women, 2) much higher in menopausal women than the estrone excretion of such women as measured by biological assay (8–10), and 3) much higher in normal men than the estrone excretion of such men as measured by biological assay (10), it may be concluded that the weakly phenolic ketones excreted include other substances in addition to estrone. It is regretted that the colorimetric assays could not be checked by biological assays.

It could be argued that these results are what one would expect, since estrone is thought to be an intermediary product in the transformation of estradiol to estriol (11). From this point of view, measurement of the urinary estrone fraction alone would give very little indication of disturbances in the production or metabolism of estrogens. Nevertheless, the present survey has been rather limited in scope. For this reason, the re-evaluation of the method is recorded in detail to facilitate subsequent work. It is hoped that a more extensive investigation of this test in patients with other disorders in the function of the estrogen-producing glands and also possibly in those with non-endocrine conditions may reveal useful clinical applications.

SUMMARY AND CONCLUSIONS

A previously described method for the determination of the weakly-phenolic-ketone (estrone) content of 24-hour human urine extracts has been re-examined and found to be adequate within limitations. Minor modifications including purification of solvents are described.

Although quantitative recovery is poor with this procedure, the recovery of crystalline estrone from standard solutions and urine pools is reasonably consistent (average 55 per cent).

The color reaction employed in this determination was found to be unsatisfactory for the colorimetric assay of estradiol and estriol.

The "estrone" excretion obtained by this method was found to correspond grossly to the physiological activity of the estrogen-producing endocrine glands of normally menstruating and pregnant women.

However, the "estrone" excretion thus obtained did not correlate with the physiological activity of the estrogen-producing glands of normal men nor with the pathological activity of the estrogen-producing glands in patients of both sexes with endocrine disorders. Diagnostic deviations were encountered only in pregnancy, which can be diagnosed more readily by other means.

It is suggested that the weakly phenolic ketones measured by this procedure may include other substances in addition to estrone.

The determination of weakly phenolic ketone (estrone) in the urine by this method has as yet no practical clinical application.

The authors are indebted to Dr. Fuller Albright, to Dr Konrad Dobriner and to Dr. Nathan B. Talbot for valuable suggestions during the course of the investigation.

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Embryology of Sexual Structure and Hermaphroditism[1]

R. R. GREENE, M.S., M.D.

From the Departments of Physiology and Pharmacology, Obstetrics and Gynecology, Northwestern University Medical School, Chicago

INTRODUCTION

THIS SECTION embodies a brief account of embryonic development of the reproductive organs but does not contain the complete details of embryonic sexual development. Detailed descriptions and controversial matters are avoided or minimized as much as possible. This embryological material, it is hoped, will aid in the understanding of the discussion of hermaphroditism, particularly the *how* and *why* in the development of the genital abnormalities. It is also hoped that the embryological material will aid in the comprehension of later development and function of the testes.

NORMAL EMBRYONIC DEVELOPMENT

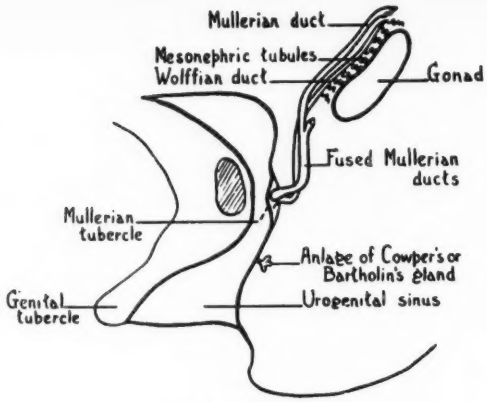
THE INDIFFERENT STAGE. The primordia or anlagen of the future sexual structures *of both sexes* are present in every early embryo. The development of sexual structures up to a certain point is essentially identical in all embryos whether they are to differentiate as males or females. During these early stages when sexual structures are characteristic of neither sex, but rather of both sexes, the embryo is said to be in the *indifferent state*. Since the embryo has the necessary primitive structures for development as either a male or a female it is said to have a *bisexual potentiality*. Diagrammatic illustrations of the indifferent sexual structures are shown in figures 1 and 2.

1. *The gonad.* The embryo, in this stage, has a bilateral primitive excretory apparatus, the mesonephros, and its excretory duct, the mesonephric or Wolffian duct which empties into the urogenital sinus. During the 6th week the epithelium along the mesio-ventral surface of the elongated mesonephros thickens. This thickening forms the *genital ridge* and the epithelium itself is known as the *germinal epithelium*. The next event in the development of the gonad is subject to some dispute. Most observers believe that there is a rapid proliferation of cells from the germinal epithelium. [See Felix (1912) and Simkins (1928) for details, and Fischel (1930) for contrary views.] This epithelial proliferation forms a bulging mass of cells of two types, the *indifferent cells* and the *primordial germ cells*. The bitter debate concerning the exact origin of the primordial germ cells [Felix (1912), Fuss (1912), Hargitt (1925), Kohno (1925), Evans and Swezy (1931), Heys (1925), and Simkins (1928)] is not pertinent to this presentation, nor is the equally acrimonious argument as to whether or not these primordial germ cells ever enter into the formation of adult ova or spermatozoa [Felix (1912), Hargitt (1925), Simkins (1928)].

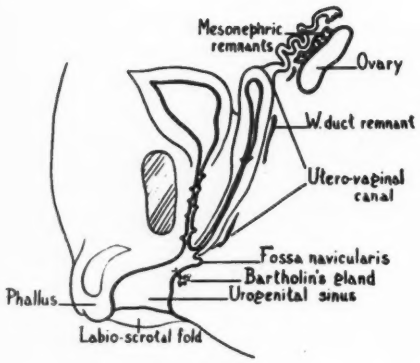
The mass of cells in the gonad anlage is then broken up into cord-like structures known as the *medullary cords*. The indifferent gonad is now formed.

2. *The rete of the gonad.* The primary origin of the rete is also subject to some dispute [von Winiwarter (1910), Felix (1912) Wilson (1926)]. It seems fairly certain, however, that it develops from a cranial portion of the gonad anlage. This portion, known as the rete blastema, also later develops into cord-like structures.

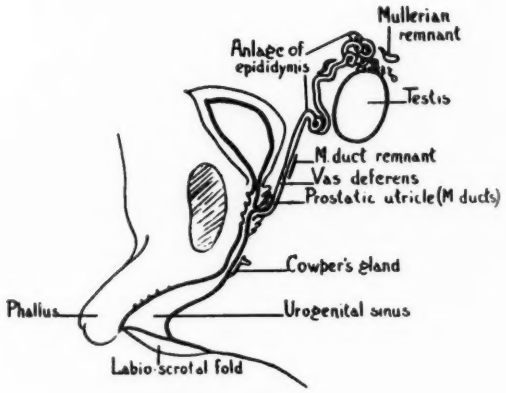
[1] This article was prepared for inclusion in a textbook in Endocrinology under the editorship of Drs. A. W. Spence, London and Fuller Albright, Boston. The publication of the book has been delayed owing to the current war emergency. The privilege of advance journal publication has been extended by the editors.



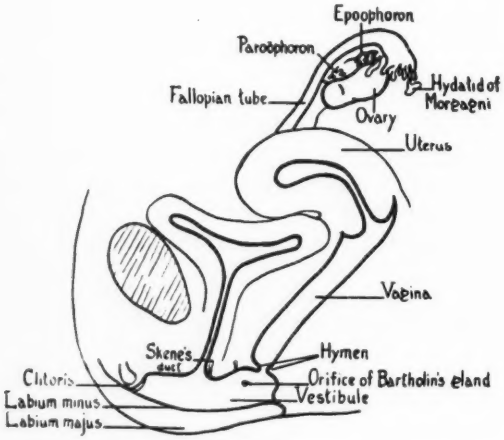
INDIFFERENT STAGE



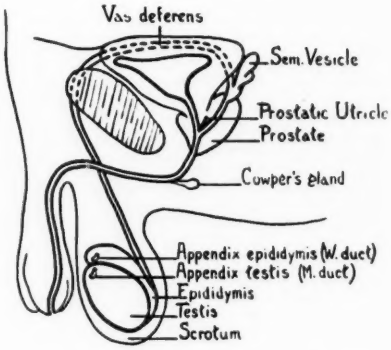
FEMALE EMBRYO



MALE EMBRYO



ADULT FEMALE



ADULT MALE RRG+MLB '11

FIG. 1

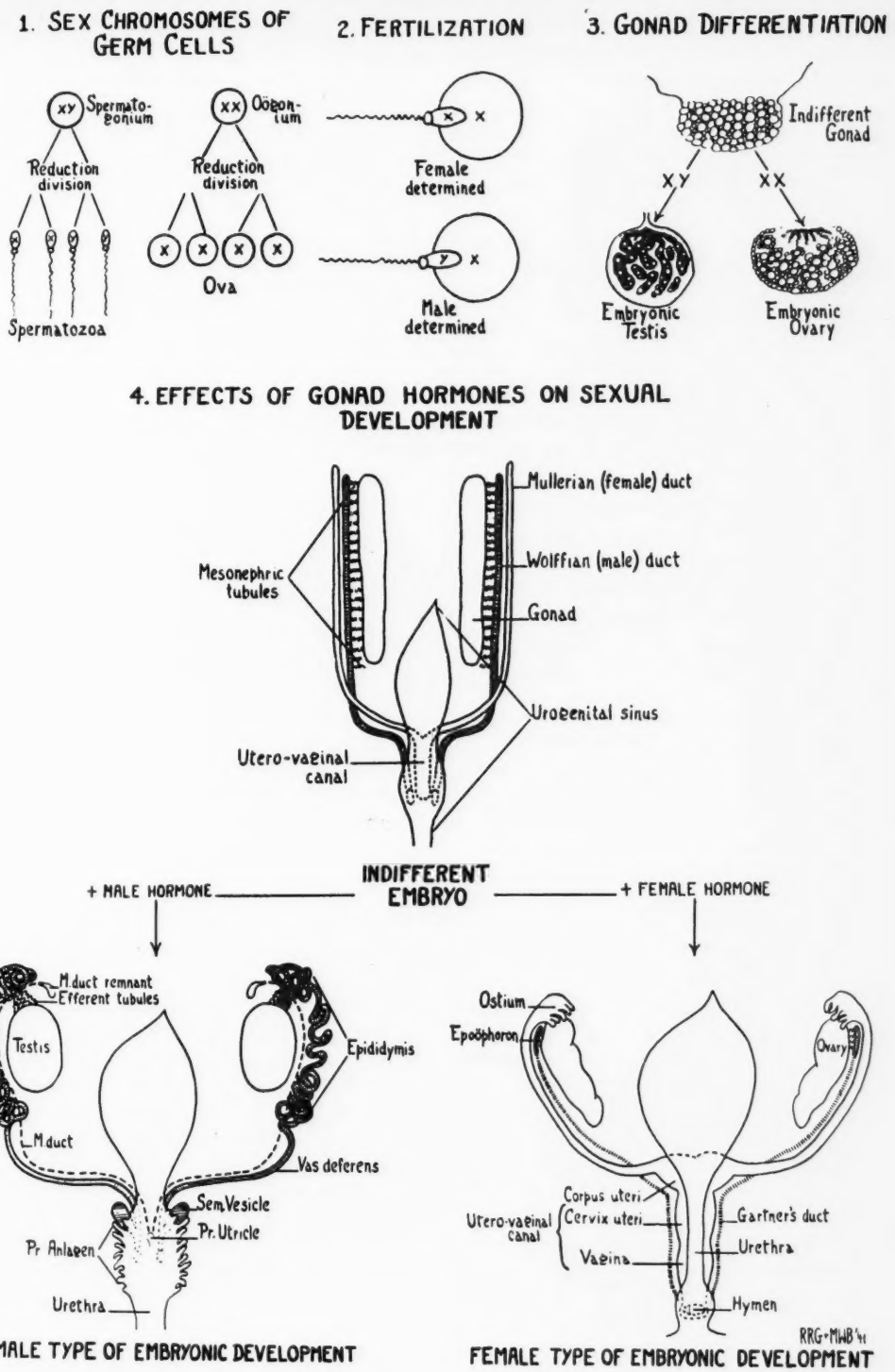

1. SEX CHROMOSOMES OF GERM CELLS
2. FERTILIZATION
3. GONAD DIFFERENTIATION
XY Spermato-gonium
Reduction division
Spermatozoa
XX Oögon-ium
Reduction division
Ova
Female determined
Male determined
Indifferent Gonad
XY
XX
Embryonic Testis
Embryonic Ovary
4. EFFECTS OF GONAD HORMONES ON SEXUAL DEVELOPMENT
Müllerian (female) duct
Wolffian (male) duct
Gonad
Mesonephric tubules
Urogenital sinus
Utero-vaginal canal
INDIFFERENT EMBRYO
+ MALE HORMONE
+ FEMALE HORMONE
M duct remnant
Efferent tubules
Testis
Epididymis
M duct
Vas deferens
Sem. Vesicle
Pr. Utricle
Pr. Anlagen
Urethra
MALE TYPE OF EMBRYONIC DEVELOPMENT
Ostium
Epoöpheron
Ovary
Corpus uteri
Utero-vaginal canal
Cervix uteri
Vagina
Gartner's duct
Urethra
Hymen
RRG-MWB '41
FEMALE TYPE OF EMBRYONIC DEVELOPMENT

FIG. 2

3. *The mesonephros and the Wolffian duct.* The *mesonephros* in the indifferent stage is an elongate structure containing numerous glomeruli each of which is connected by a mesonephric tubule to the *mesonephric or Wolffian duct.* The Wolffian duct extends along the entire length of the mesonephros. From the caudal end of the mesonephros the Wolffian duct courses for a short distance towards the midline. It then resumes a caudal course in a structure known as the *genital cord* and eventually empties into the urogenital sinus. The Wolffian ducts and some of the mesonephric tubules play a very important role in the development of the male sperm-conducting structures, but regress or remain as non-functional remnants in the female.

4. *The Mullerian duct.* The Mullerian duct begins at the cranial end of the mesonephros as an invagination of the coelomic epithelium into the mesenchyme to form a trough or deep furrow. The cranial end of this furrow remains open. The caudal end roofs over and a cord of epithelial cells grows out from it, extending caudalwards parallel to the Wolffian duct. In the genital cord the two Mullerian ducts are mesial to the two Wolffian ducts and lie very close together. They usually fuse with each other before reaching the urogenital sinus, mesial to the openings of the two Wolffian ducts.

5. *The urogenital sinus and the external genitalia.* The urogenital sinus, in the indifferent phase, is a relatively simple, elongated, hollow structure. Most of the bladder is formed from its cranial portion. At about its mid-portion the dorsal wall bulges inwards. This area, where the Mullerian ducts make contact with the sinus, is called the *Mullerian tubercle.* The caudal third of the urogenital sinus is known as the phallic portion and extends to the tip of the anlage of the phallus. The latter is a fairly large structure, the genital tubercle. The ectodermal epithelium along the caudal surface of the genital tubercle is fused with the floor of the urogenital sinus forming the *urogenital plate.* On either side of the genital tubercle are swellings, the two *labioscrotal folds.*

Descriptions of the sexually indifferent phase of embryonic development usually give the impression that the various structures all exist at the same time. This is not quite true. The indifferent stage must be considered as having elastic limits. For example, the beginning differentiation of the gonad into a testis can be recognized before the Mullerian ducts reach the urogenital sinus. Facts such as this, however, do not invalidate the original concept that *the embryo, whatever its genetic sex, has the necessary* primordia for development as either a male or female.

SEXUAL DIFFERENTIATION. Sexual differentiation is, to a certain extent, less complicated in the male than in the female. In this regard then the female shows a greater degree of development than the male. Differentiation of the male and female sexual structures are presented diagrammatically in figures 1 and 2. The accompanying table lists the adult male and female sexual structures derived from the various indifferent sexual structures.

1. *Gonads. In the genetic male,* the gonad starts to differentiate early (7 weeks) by development of the basic primordia present in the indifferent state. The medullary cords become well separated and surrounded by connective tissue. At the same time a well developed connective tissue *tunica albuginea* separates the medullary cords from the germinal epithelium. The large germinal epithelial cells regress to their original low mesothelial type and finally resemble the peritoneal epithelial cells.

The peripheral portions of the medullary cords eventually form the *convoluted seminiferous tubules.* The more central portions (near the hilum) become the *tubuli recti* of the testis. These latter establish communications with the *rete testis* which has been organized first into cords and then tubules.

In the genetic female, differentiation of the gonad starts much later than in the male (about 10 weeks). The primordial gonadal structures, the medullary cords, which are so important in the male, play no part in the formation of the ovary. It is developed from an added cortex of the gonad. This is formed by further proliferation of the germinal epithelium. This cortical proliferation, similar to the primary or medullary proliferation, consists of two types of cells, the primordial germ cells and the indifferent cells. *Cortical cords* are formed secondarily by a breaking up of the

HOMOLOGOUS STRUCTURES IN MALE AND FEMALE
Non-functional structures in italics

Indifferent Embryo	Female	Male
Gonad A—Germinal epithelium. B—Medulla. 1—Cords. 2—rete blastema C—Cortex.	Ovary A—Germinal epithelium. B— 1—none (usually). 2—rete ovarii C—Definitive ovary.	Testis A—Visceral lamina of tunica vaginalis. B—Definitive testis. 1—Contorted and straight tubules of testis. 2—Rete testis. C—None.
Mesonephric Tubules A—Cranial group. B—Caudal group	A—*Epo-ophoron.* B—*Paro-ophoron.*	A—Efferent tubules (conus epididymis). B—*Paradidymis, aberrant tubules (appendix epididymis).*
Mesonephric (Wolffian) Duct A—Cranial. B—Middle. C—Caudal.	A—Longitudinal tubule of epo2phoron. B—*Gartner's duct.* C—*Gartner's duct.*	A—Epididymis. B—Vas deferens. C—Seminal vesicle and ejaculatory duct.
Müllerian Duct A—Cranial B—Middle. C—Caudal.	A—Fallopian tubes (oviducts) *Hydatid of Morgagni.* B—Fundus uteri. C—Cervix uteri and vagina.	A—*Appendix testis (Hydatid of M.)* B—None. C—*Vagina masculina (prostatic utricle).*
Urogenital Sinus A—Cranial. B—Caudal (phallic).	A—1—Part of bladder. 2—Urethra. 3—Paraurethral (Skene's) glands. 4—Vestibular (Bartholin's) glands. B—1—Vaginal vestibule. Fossa navicularis. 2—Inner surface of labia minora. 3—Minor vestibular glands.	A—1—Part of bladder. 2—Prostatic urethra. 3—Prostate. 4—Cowper's glands. B—1—Membranous urethra. 2—Cavernous urethra. 3—Glands of Littre (peri-urethral glands).
Genital Tubercle	1—Clitoris. 2—Prepuce of clitoris.	1—Glans penis. 2—Prepuce.
Labio-Scrotal Swellings	Labia majora.	Scrotum.
Inguinal Fold A—Cranial. B—Caudal.	A—Ovarian ligament. B—Round ligament.	A—Gubernacular ligament. B—Gubernaculum.

solid masses of proliferating germinal epithelial cells. Later the cords are also broken up and there is a rearrangement of the indifferent cells around the germ cells to form the *primitive follicles*. While this process has been going on, the medulla of the gonad has been degenerating. Remnants of medullary cords are only rarely found in adult ovaries. The rete of the gonad, however, undergoes some differtiation in that cords and then tubules are formed (Wilson, 1926). While this development is much less extensive than in the male, *rete tubules* can usually be found in the hilum of the normal adult ovary.

2. *Gonad homologies.* The *Sertoli or supporting cells* of the seminiferous tubules are considered to be derived originally from the indifferent germinal epithelial cells in the germinal cords. For this reason they are considered as homologous to the similarly derived *granulosa or follicular cells* of the ovarian follicle. It is also claimed that the *theca interna* cells of the ovary have the same origin as the *testicular interstitial cells* (cells of Leydig), both originating from indifferent germinal epithelial cells that were left isolated in the connective tissue during the process of germinal cord formation. These cell homologies are the basis of certain theories regarding hormone formation. It is known that ovaries may produce androgens and that testes may produce estrogens. It is theorized that the homologous ovarian granulosa cells and testicular Sertoli cells both produce estrogens. It is also theorized that ovarian thecal cells (theca lutein cells) produce androgens as do the homologous interstitial cells of

the testis. There is some evidence supporting these theories, but unfortunately there is equally good evidence challenging their validity.

3. *Descent of the gonads.* In the early embryonic state the upper pole of the gonad is at a very high level. Later in development the fetal ovary and testis lie at a lower level, just inside the true pelvis. This descent of the gonad is more apparent than real. It is largely due to different rates of growth in various parts of the body. When first developed, the gonad is about one-half the total length of the embryo. Later it is relatively very much smaller. The *diaphragmatic ligament* of the mesonephros which originally fixes the cranial portion of the gonad degenerates very early. The *inguinal ligament* of the mesonephros which fixes the caudal pole of the gonad remains, however, for future differentiation. Since the body in general is growing at a much more rapid rate than the gonad and the gonad is fixed at a constant caudal point, it apparently descends. Some degeneration of cranial portions of the gonad may aid in this apparent descent (Felix, 1912).

4. *Gubernaculum, round and ovarian ligaments.* The previously mentioned inguinal ligament undergoes important differentiation specific to each sex. In the male it forms the *gubernaculum* and the *gubernacular ligament.* In the female it forms the *ovarian and round ligaments.*

The true descent of the gonad in the male is to be discussed separately. In the female the caudal end of the inguinal ligament becomes fixed in the labio-scrotal folds (as it does in the male). Descent of the ovary, however, is prevented by growth of the uterus. This rapidly growing structure divides the inguinal ligament into two parts: the cranial ovarian ligament and the caudal round ligament. The ovarian ligament fixes the ovary in the adult position.

5. *Mesonephric tubules and the Wolffian duct.* Most of the mesonephric tubules degenerate early. At about 3 months in both sexes 8 to 15 of the more cranial tubules establish continuity with the rete testis or rete ovarii. The rete-tubule junctions are usually more numerous and more definite in the male than in the female.

In the male, these cranial mesonephric tubules hypertrophy and form the *efferent tubules* of the testis (conus epididymis) which communicate with the highly convoluted cranial portion of the Wolffian duct (*epididymis*). The middle part of the Wolffian duct in the male forms the *vas deferens.* An outpouching from the lower part of the duct forms the *seminal vesicle* and caudad to this structure the remainder of the duct becomes the *ejaculatory duct.*

In the female, however, further development of the mesonephric tubules is minimal, although homologues of the efferent tubules usually remain as the transverse tubules of the epoöphoron. Most of the Wolffian duct disappears, although the cranial portion usually remains as the longitudinal tubule of the epoöphoron. A persistent Wolffian duct in the adult female is known as Gartner's duct. It may be complete, but is more frequently incomplete or interrupted in its course. The orifices of Gartner's duct, if present, are located on the external surface of the hymen.

6. *The Müllerian duct.* In the male the Müllerian duct almost completely degenerates. Only the most caudal portion persists as the *prostatic utricle* or *vagina masculina.* In the female the cranial half of each Müllerian duct forms the *Fallopian tube.* The middle or horizontal portions of the ducts are incorporated in the developing uterus to form its *fundus.* The fused caudal part of the two ducts is known as the utero-vaginal canal. The upper portion of this structure differentiates into the *cervical portion* of the uterus and the lower portion takes part in the formation of the *vagina.* How much of the vagina is derived from the Müllerian ducts is subject to much controversy.

7. *The vagina.* Lack of space precludes discussion of the many diverse theories regarding the formation of the human vagina [Wood-Jones (1904), Hart (1901), Nagel (1891), Felix (1912), Meyer (1909, 1934, 1937abc), Bloomfield and Frazer (1927), Hunter (1930), Koff (1930 and 1933), and Spuler (1930)]. It seems certain that the upper vagina is purely Müllerian in origin. It seems probable that the lower vagina down to and including the inner surface of the hymen is also of Müllerian origin. It also seems probable that the hymen repre-

sents the original point of junction of the Müllerian ducts with the urogenital sinus (Müllerian tubercle). These statements, however, are the personal opinions of this author, based on examination of the evidence presented by various workers. It is possible that future investigations may prove this interpretation to be erroneous.

8. *The urogenital sinus.* The most cranial part of the urogenital sinus forms most of the bladder in both sexes. Only a small part of the remainder of the urogenital sinus is utilized to form the urethra in the female. All of the remainder is, however, so employed in the male and forms prostatic, membranous and cavernous portions of the male urethra.

Near the end of the 3rd month, multiple epithelial outgrowths develop from the urogenital sinus. The most cranial groups of outgrowths form the *prostate* in the male. In the female most of these cranial outgrowths regress. A few remain, however, as *Skene's ducts* (*paraurethral ducts*). At about the mid portion of the urogenital sinus paired glandular outgrowths develop. These become *Cowper's glands* in the male and *Bartholin's* (*vestibular*) *glands* in the female. The caudal outgrowths which are smaller and less numerous than the cranial ones form the glands of *Littre* in the male. In the female most of these degenerate, but a few may remain as minor vestibular glands (Wilson, 1926).

As previously stated, only a portion of the urogenital sinus is utilized to form the urethra in the female. The lower part of the urogenital sinus (approximately one-half) lags in longitudinal growth and later forms the *vaginal vestibule* or the area from the external surface of the hymen to the free borders of the labia minora.

9. *External genitalia.* The early anlagen of the external genitalia, the genital tubercle and the labio-scrotal folds, are identical in both sexes. The urogenital plate which lies on the caudal surface of the genital tubercle splits open in the mid-line (*urethral groove*) forming the primitive urogenital ostium. In the female it remains open and is represented later by the free borders of the *labia minora.* In the male, however, it roofs over again to form the *cavernous urethra.*

In both sexes the genital tubercle grows to form the phallus. In the female a minimal degree of further growth and development forms the *clitoris.* In the male more extensive growth and development takes place and the *penis* is formed.

In the female there is only a moderate development of the labio-scrotal folds to form the *labia majora.* In the male growth is more extensive. Eventually the labio-scrotal folds meet in the mid-line to fuse and form the *scrotum.*

CONTROL OF SEXUAL DEVELOPMENT. 1. *Genic factors.* The sex of the embryo is generally believed to be determined at its inception. The determining factors are considered to be specific by multiple genes located almost exclusively in the so-called sex chromosomes. The sex determining chromosomes of the human are known as the X and Y chromosomes. In the cells of the female body there are two X chromosomes. In the male, however, there is one X and one Y chromosome. In the maturation of the female germ cells the total number of chromosomes is halved so that, as a result, each mature ovum has one X chromosome. In the maturation of the male germ cells a similar reduction process occurs so that one-half of the mature spermatozoa have only an X chromosome, the other half have only a Y chromosome. If the ovum is fertilized by an X bearing sperm, the resulting embryo has two X chromosomes which together contain the necessary genes to initiate female development. If, however, the ovum is fertilized by a Y bearing sperm, the resulting embryo has the X-Y chromosome combination which initiates male type of development.

2. *Other factors in sexual development.* The genes do not possess an absolute control over the sex of an individual. The genes are chemical substances and exert their effects through chemical processes. There is ample evidence in lower animals that other physico-chemical factors, such as temperature, delayed fertilization, and state of hydration, can modify or even reverse the processes of sexual development [see Willier (1939) and Witschi (1939) for details]. There is also more recent evidence that the sex hormones have profound effects on embryonic sexual development. This has been directly demonstrated not only in the

lower animals, but also in mammals such as the rat (Greene and Ivy, 1937, and Greene, Burrill and Ivy, 1938abcd, 1939abcd, 1940ab), the mouse (Raynaud, 1938abcd, 1939abcd), the guinea pig (Dantchakoff 1936, 1937ab, 1938 abcd), and the monkey (Morse and Van Wagenen, 1940).

3. *Control of normal sexual development.* The most reasonable supposition at the present time is that the genic factors initiate the chain of processes causing differentiation of male or female structures. Directly or indirectly the genes cause organization of the indifferent gonad into a testis or an ovary. The differentiated gonad produces a sex hormone or sex hormone-like substance. These substances, in turn, cause the other indifferent structures to differentiate into sexual structures characteristic of the male or of the female.

The reader is not to infer that this concept of normal sexual differentiation is an established fact nor that it is universally accepted as true. It should be regarded as a theory which in the opinion of the author conforms best with the available observations and experimental data on this subject.

The contention that sex hormones have nothing to do with embryonic sexual development seems improbable in view of the facts that embryonic gonads are known to produce sex hormones (Atumi, 1939, Womack and Koch, 1930), and that experimental work has shown that sex hormones have unquestionable effects on embryonic sexual development.

The *monhormonic* theory of sexual development postulates that development in the male is controlled by male hormone, but that the female hormone has no effect and, therefore, female differentiation takes place because male hormone is absent. This theory seems unlikely since it has been shown in experimental animals that the female hormone does cause female type of sexual differentiation in the male embryo (Greene, Burrill and Ivy, 1938c, 1939abd and 1940a, and Raynaud, 1939abcd). Proponents of the monhormonic theory argue that if the female hormone has any effect on sexual differentiation, all human males would be feminized by the tremendous concentration of female hormone present in the pregnant female. This argument has neglected the fact that this "tremendous" concentration of female hormone is present during the latter part of pregnancy, *after* sexual differentiation has taken place. Another fact which has been overlooked in this argument is that androgens are also present in the pregnant female (Womack and Koch, 1930). Androgens, in terms of the usual biological units, are much more potent than estrogens in modifying embryonic sexual structures (Greene, Burrill and Ivy, 1940b). Androgens and estrogens are mutually antagonistic and one can nullify the effects of the other on embryonic sexual structures (Greene, Burrill and Ivy, 1940b). It seems quite probable that sufficient androgens are produced by the pregnant female to nullify any possible effects of maternal estrogens on the embryo and that *normally* the maternal hormones have no effect on embryonic sexual development.

In summary, a reasonable theory in accord with the known facts may be presented to explain the control of normal sexual differentiation. The genic factors set the course for normal sexual differentiation in the male or female direction while the sex hormones steer the course by their influence on the processes involved. These sex hormones are not those produced by the mother, but those produced by the fetus itself. Deviations from the normal course of sexual development, the factors involved, and the resulting abnormalities will now be discussed.

INTERSEXUALITY OR HERMAPHRODITISM

The term *hermaphroditism* has several meanings. Strictly speaking it refers to a condition in which the sexual structures of both sexes are present in one individual and implies that such an individual can carry on all the functions characteristic of both sexes. There are no authentic records of the occurrence of such individuals. The term *true hermaphrodite* is commonly used to refer to an individual who has both an ovary and a testis or ovarian and testicular tissues combined in the same gonad. When an individual has gonads of one sex and all other sexual structures of the opposite sex the term *pseudo-hermaphrodite* is used. This same term is also applied to the more common individual having gonads of one sex and variable mixtures of other male and female struc-

tures. The term *intersex* is, in some respects, preferable. When used in this discussion, it refers to any of the so-called true or pseudo-hermaphroditic types.

THEORIES. There are many theories purporting to explain intersexuality. These theories will not be reviewed in detail, although a few comments seem apropos.

Goldschmidt formulated a rather complicated theory on the basis of his experimental work with insects (Goldschmidt, 1931). This theory is frequently accepted as valid in the clinical literature, and is interpreted to mean that all true or pseudo-hermaphrodites are genetic females. The accuracy of this interpretation seems most doubtful in view of more recent observations.

In animals such as fish, amphibia and birds, complete or partial sex reversals have been produced by administration of large amounts of female hormone to the male embryo. In the mammal, the development of all sexual structures of the male embryo, except the gonad, has been feminized by a similar procedure. Witschi has presented excellent evidence that those individuals usually designated as male pseudo-hermaphrodites represent feminized genetic males (Witschi, 1939).

McCahey has asserted that the status of the Müllerian-duct derivatives is the real index as to the genetic sex of true or pseudo-hermaphrodites (1938 and 1939). Thus, for example, the presence of Müllerian duct derivatives "no matter how vestigial" in a pseudo-hermaphrodite with normal testes means that he is a genetic female. Pursuing McCahey's assumption to its logical conclusion, there could be no true genetic males in the human race since they all have vestigial Müllerian derivatives. The prostatic utricle is a Müllerian vestige which is present in every normal male and the appendix testis is another Müllerian remnant which is frequently present.

It seems doubtful whether any one theory or any one mechanism will explain all cases of human intersexuality. It is a little out of the question to do experimental work on modifying embryonic sexual development in the human. Unfortunately it is also hazardous to assume that the human necessarily reacts to genic sexual factors as does the insect or to hormonal sexual factors as does the laboratory mammal. Yet, with the present state of our knowledge, such assumptions are necessary to arrive at any understanding or *tentative* explanation of these matters. The following statements are presented, therefore, as being probably true, but should be accepted with some mental reservation as to their eventual validity.

Human intersexuality may be due to either genic or to hormonal factors. In some individuals it is difficult to determine the causal factor. In others the nature of the causal factor, whether genic or endocrine, seems obvious. There is certainly excellent reason to believe that genetic male embryos as well as genetic female embryos are subject to intersexual changes.

In those few individuals having a separate and distinct ovary and testis, a genic derangement is probably at fault. In individuals having ovotestes it is difficult to state which is the fundamental causal factor. If, due to genic derangements, both testicular and ovarian tissue differentiates, probably both male and female hormones are produced, although not necessarily in equivalent quantities. Further development of the embryo would then be conditioned by the hormone which predominates. Thus, even though the fundamental fault is genic, hormonal abnormalities would be produced which would further modify sexual development.

If the evidence that development of the embryonic gonad itself can be modified by sex hormones in lower animals and possibly even in the guinea pig (Dantchakoff, 1939) and the free-martin of cattle (Lillie, 1917) can be applied to the human, production of ovotestes may be due to a purely hormonal cause. The source of the hormone in such cases would be extra-gonadal. Abnormal production by the maternal organism of an excess of one of the sex hormones might thus cause the development of an intersex with ovotestes.

In the individuals having gonads of one sex and part or all of the remaining structures characteristic of the other sex, the causal mechanism is probably completely hormonal. This would be due to excess maternal production of one of the hormones or even to an embryonic extra-gonadal production of excess sex hor-

mone. An hormonal cause for this type of intersexuality has particularly strong support from evidence obtained in mammals. In our own work with the rat, for example, genetic males with testes but most other sexual structures characteristic of females have been produced and also the converse, genetic females with ovaries and most other structures characteristic of males. To produce these changes very large doses of sex hormones are given to rats during certain stages of pregnancy. If estrogens are used, the male offspring are markedly feminized except for the gonads (Greene, Burrill and Ivy, 1938c, 1939abd, 1940a). If androgens are used, the female offspring are masculinized except for the gonads (Greene and Ivy, Greene, Burrill and Ivy 1937; 1938abd, 1939c). The fact that we have not altered differentiation of the gonad must not, however, be interpreted as evidence that hormones cannot modify gonads in rats. Attempts to modify gonads by giving higher doses of sex hormones earlier in pregnancy have caused fetal deaths. The data does indicate, however, that hormones can modify development of sexual accessories under conditions in which they do not modify the gonads. The lack of effect on the gonad may be quantitative, i.e., insufficient dosage, and the time factor may also be involved (Burrill and Greene, 1939). As stated before, the indifferent gonads undergo sexual differentiation before the other sexual structures. *Once differentiation of a sexual structure has taken place, its developmental trend can no longer be reversed.*

It is commonly accepted in the clinical literature that the changes in genetic female intersexes (ovaries in combination with some or all male sexual structures) have been caused by androgens produced in the embryonic adrenals. In some individuals of this type, this adrenal androgen production continues in post-natal life to produce a post-natal type of masculinization (further growth of clitoris, growth of body and facial hair, and voice changes). Contrary to the general impression, there is no good reason to believe that all intersexes with ovaries and other structures of the male type are caused by excess androgens from the embryonic adrenals. Many of these individuals show no evidence of the adreno-genital syndrome at any age. It is just as logical to believe, but equally difficult to prove, that the cause in some cases may be an excessive production of maternal androgens during the crucial stages of embryonic sexual development.

As has been indicated, intersexes of the pseudo-hermaphroditic type may have some sexual accessories characteristic of one sex and others characteristic of the other sex. For example, there are individuals in whom typical female external genitalia are found in combination with testes, well developed epididymides and vasa deferentia. Such abnormalities could be produced by a temporary excess of maternal estrogens. The normal effects of the embryonic testicular hormone on sexual structures undergoing differentiation at the time of this temporary excess would be modified or reversed by the maternally produced estrogens. Sexual structures which differentiate before or after the temporary excess would escape the influence of the estrogens and therefore would develop in the normal male manner. Likewise a temporary excess of androgens during embryonic development could produce combinations of male and female characteristics such as a male type of phallus (generally hypospadiac) and absence of a lower vagina in an individual with an otherwise female type of reproductive tract (ovaries, fallopian tubes and uterus). The temporary excess of androgen (whether, in this instance, maternal or fetal in origin) inhibited normal development of the lower vagina and, at the same time, stimulated the development of the phallus in the male direction. The uterus and tubes had differentiated, however, under the influence of the embryonic ovaries *previous* to the sudden influence of androgens.

CLINICAL CONSIDERATIONS

INCIDENCE. Space limitations prevent any survey of the clinical literature on the subject of hermaphroditism. Exhaustive treatises on the subject have been published by von Neugebauer (1908) and by Young (1937).

The frequency of intersexual changes of one type or another in the human is greater than is generally assumed. Von Neugebauer, in 1908, presented the records for 2072 cases. Young places the incidence at 1 in 1000 individuals.

He does not give the source for this estimate and possibly it is too high. Certainly, the ordinary physician does not see many such individuals per year. It must be remembered that a good many cases of intersexuality have become known only at autopsy. It is quite probable that many individuals with intersexual genital abnormalities never consult a physician.

CLASSIFICATION. Many classifications have been formulated for the various types of human intersexuality. Most of these classifications are very complex and will not be discussed here. The various types will be considered under three major groups: first, those individuals having both an ovary and a testis; second, those individuals having ovotestes; and third, those individuals having gonads of one sex and some or all the accessory sexual structures of the other sex.

1. *Intersexes with separate ovary and testis.* This type of intersexuality in the human is most rare. Young accepts only three such cases as authentic, i.e., proven by microscopic examination of the gonads. Each of these individuals had a testis on the right side and an ovary on the left. He has not included, however, an intersex reported by Sheppard (1920). This individual was stated to have bilateral ovaries in the normal ovarian position and bilateral testes in the scrotum. Such an individual would indeed be unique. These findings were made during anatomical dissection of a poorly preserved cadaver. Unfortunately, the histological findings, as judged by the photomicrographs, are obscured by post-mortem necrosis. It would be debatable, then, as to whether this should be accepted as a microscopically proven case of an intersex with both ovaries and testes. Jasienski (1938) and others have also reported intersexes of this type. Their diagnoses, however, were made on the basis of gross appearance of the gonads. Since the gonads of intersexes are often malformed, underdeveloped and grossly atypical, a diagnosis made by inspection only may not be accurate.

As previously mentioned, the cause of this particular type of intersexuality is probably genic. These individuals may be analogous to the insect gynandromorphs in which the chromosomal complex is of male type (X-Y) in one part of the body and female type (X-X) in another part [see Bridges (1939) for further discussion].

2. *Intersexes with ovotestes.* The majority of the reported "true hermaphrodites" fall into this group. In 1937, Young accepted 17 reports of intersexes with ovotestes as microscopically proven. Three more acceptable cases reported subsequently have been noted in the American literature (Doss and Priestly, 1940; Kell, Mathews and Backman, 1939; Smith, Mack and Murray, 1939) and also two cases whose microscopic validity seems doubtful (Reinberger and Simkins, 1938 and Young, 1940). The details on still another case will be reported by Drs. H. O. Jones and J. I. Brewer. It is of interest to note that this latter individual had a normal corpus luteum and normal ovarian follicles, plus seminiferous tubules with spermatogonia and Sertoli cells and also well developed interstitial cells (cells of Leydig). In spite of the presence of these latter supposed male hormone-producing cells, the habitus, breast development, hair distribution, mannerisms and sexual desires were those of the normal female.

The accessory sexual structures of this type of intersex have shown a great deal of variability in the reported cases. The external genitalia have varied from normal female to normal male in type, although the majority have an hypospadiac penis-like organ with a vagina which empties into the urethra. A uterus was present in most of these individuals, but none was present in the Jones and Brewer intersex. In general, the findings in the sexual structures and the psychic organization show only a fair correlation with the relative amounts of ovarian or testicular tissue present.

The possible causes of this type of intersexuality have already been mentioned. With the embryonic ovotestis producing both male and female hormone, the variable findings in the sexual accessories of these interesexes seems reasonable.

3. *Intersexes with the gonads of one sex.* As previously indicated, the relative incidence of intersexuality is much higher than is usually recognized. The vast majority of such individuals have gonads of one sex and some or all sexual accessories of the other sex. This group

would include the third type to be discussed, those individuals more commonly known as pseudo-hermaphrodites. It might properly also include a number of individuals who are not generally considered intersexes, but who certainly show genital abnormalities of the intersexed type. By this is meant individuals in whom almost all sexual structures are normal, but one or more structures have differentiated in a manner characteristic of the opposite sex. This would include, for example, all females with definite prostatic tissue and most males with hypospadias, a condition that has been produced experimentally in the rat with female hormone (Greene, Burrill and Ivy, 1940a).

The more interesting members of this class have had more extensive interference with sexual differentiation. As would be inferred from the discussion on the cause of such conditions, bizarre combinations of male and female sexual structures may be found. The most perplexing medical problems are those individuals with gonads of one sex and external genitalia of another. This author has seen two male intersexes with normal female external genitalia. The true condition was diagnosed in one case when an operation in childhood for inguinal hernias revealed the presence of testes. In the other case, changing voice, growth of facial hair and an enlarging clitoris caused investigation and discovery of the abnormality.

As a rule, in intersexes with ovaries, the masculinization of the external genitalia is not quite complete in that an hypospadiac penis is present. In the two children of this type seen by the author, such was the case. It should be mentioned that in neither of these two children was there any evidence of the adreno-genital syndrome.

TREATMENT. The actual treatment of intersexuality is largely surgical. Surgical technique lies outside the scope of this presentation and will not be discussed.

The question of exactly what should be done for these individuals has been the subject of some debate. Our knowledge on the subject of human intersexuality is too meager to afford many positive opinions. Nevertheless, the author is very positive in this opinion: the physician should make every effort to render it possible for the intersex to function as well as possible in accordance with his or her *emotions or desires*. The genetic sex of the individual is of scientific interest, but unfortunately does not always conform with the psychic sex of the individual. The use of surgery to alter sexual structures to conform with psychic sexuality is often successful. On the other hand, even if the patient would permit it, attempts to alter the psychic sex to conform with the genetic sex are usually unsuccessful.

The author is also opinionated on another subject: these individuals are too frequently castrated. While biopsies of gonads for diagnostic purposes do little harm, routine removal of gonadal tissue often does. This is particularly true in children. The gonads in these intersexes do not of necessity produce a preponderance of hormone characteristic of the genetic sex. Young, Quinby (1922) and others cited many adult intersexes in which the converse is true. If, after maturity, the gonads produce hormone effects judged as undesirable by both the patient and the physician, castration should no doubt be done and substitutional hormone therapy provided. The frequency of gonad tumors (dysgerminomas) is greater in these intersexes than in normal individuals. They should therefore be warned of this fact and carefully observed.

Hormonal therapy is of value in a few selected cases as an adjunct to surgery. For example, one of the male intersexes with female external genitalia seen by the author and Dr. Ivy has had two plastic operations by Dr. Harry Culver to date. He is to have a further operation, but in the meantime is getting very large doses of male hormone (50 mg. of testosterone propionate daily) to develop the phallus to penis-like proportions. Treatment is being given intermittently (during alternate months) to minimize depression of his own gonadal function.

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Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor: D. A. McGINTY. *Collaborators:* ISRAEL BRAM, RUCKER CLEVELAND, JOHN W. EVERETT, ANNE FORBES, ROBERT B. GREENBLATT, E. C. HAMBLEN, CHARLES W. HOOKER, R. G. HOSKINS, THOMAS H. MCGAVACK, A. E. MEYER, DORIS PHELPS, J. P. PRATT, E. C. REIFENSTEIN, JR., PATRICIA H. SMITH, RUTH ST. JOHN, CHARLES W. TURNER, AND HAROLD WOOSTER.

ADRENALS

RUCKER, M. P.

Correspondence: constriction rings and epinephrine. *Am J. Obst. and Gynec.* 46: 331. 1943.

The author refutes the statement of Brown and Wilder: (*Am. J. Obst. and Gynec.* 45: 659. 1943) that epinephrine is of no use in relieving contraction rings of the uterus. The above authors only used one-half minim dosage. In 91 out of 93 patients the present author has felt, with his hand in the uterus, the contraction ring give way some few minutes after the intramuscular administration of five to six minims of epinephrine.—*E.C.H.*

ENDOCRINE GENERAL

BEAUMONT, D. M., AND J. D. ROBERTSON

Pituitary hypothyroidism with impaired renal function. *Brit. Med. J.* 2: 356. 1943.

A case of pituitary hypothyroidism is described which presented the following signs and symptoms. (1) Renal impairment:—This was the most prominent sign, and at first suggested a diagnosis of "latent nephritis." The urea clearance and water elimination were diminished, and there was a urine of low specific gravity. (2) Hypothyroidism:—This is to be distinguished from the typical primary myxoedema, in which the blood cholesterol is usually raised. (3) Cachexia. (4) Adrenocortical insufficiency. (5) Hypogonadism. (6) Decreased diabetogenic activity.

Treatment with sodium chloride and the hormones of the thyroid, gonads, and adrenal brought about a marked improvement in the clinical condition and restored the renal function to normal.—*R.B.G.*

COHN, S. AND J. I. KUSHNER

Struma ovarii. *Am. J. Obst. and Gynec.* 47: 421. 1944.

A brief case report of a struma ovarii occurring in a dermoid cystoma is given. There were no signs of thyroid disease and the basal metabolic rate was plus five.—*E.C.H.*

FRANK, R. T.

Advances in endocrine therapy. *Am. J. Obst. and Gynec.* 47: 561. 1944.

This is a review article in which the author is didactic. With only one exception, the recommendations are limited to fully authenticated and proved applications. The treatments of amenorrhea, excessive bleeding and dysmenorrhea are considered briefly. The author stresses the commonest error in endocrine therapy is that of employing hormones when no deficiency exists. —*E.C.H.*

FRIED, P. H.

The influence of the hormonal steroids on urinary tract disturbances associated with gynecologic disorders. *Am. J. Obst. and Gynec.* 46: 459. 1943.

This is a review of the effect of the hormonal steroids (androgens, the ovarian hormones and desoxycorticosterone acetate) on the urinary system of experimental animals and humans and a consideration of the therapeutic uses for, and dangers in the use of these hormones in urinary tract disturbances in women.—*E.C.H.*

GREENE, H. J. AND W. A. LAPP

Adrenal rest tumor of the ovary. *Am. J. Obst. and Gynec.* 47: 63. 1944.

An adrenal rest tumor of the ovary was found in a 29 year old colored woman who had not menstruated for four years. Following operation, menses returned and there was regression of the masculinization during the follow-up period of three years.—*E.C.H.*

KANNERSTEIN, M., C. R. BROWN AND J. A. ROSEN

Hypernephroma of the ovary. *Am. J. Obst. and Gynec.* 46: 290. 1943.

The authors state that this instance constitutes the third, possibly the fourth, reported one of metastasis of a renal hypernephroma in an ovary.—*E.C.H.*

KEPLER, E. J., M. B. DOCKERTY AND J. T PRIESTLEY

Adrenal-like ovarian tumor associated with Cushing's syndrome (so-called masculinovoblastoma, luteoma, hypernephroma, adrenal cortical carcinoma of the ovary). *Am. J. Obst. and Gynec.* 47: 43. 1944.

A detailed case report is given of a 16 year old girl in whom a preoperative diagnosis of adrenal cortical tumor of the ovary was made. This was confirmed at operation.

Clinical and pathologic data are summarized for this patient and for the 13 additional patients previously reported in the literature. Histogenesis is discussed. The authors favor the theory of adrenal cortical rests.—*E.C.H.*

MAZER, C. AND R. GREENBERG

Low-dosage irradiation in the treatment of amenorrhea. *Am. J. Obst. and Gynec.* 46: 648. 1943.

Low-dosage irradiation was applied to the pituitary gland and the ovary of 92 women who experienced varying degrees of infrequent bleeding or no bleeding. Their ages varied from 17 to 36 years. The bleeding history and the response to therapy are given as follows: of 10 patients who had not bled for 18 months to six years, five have been bleeding regularly for one to five years; of 12 patients who bled every six months, eight have been normally cyclic during a follow-up period averaging two and one half years; of 68 patients who bled every two to four months, 52 have had regular cycles for one to five years. One patient with a delayed menarche (18 years of age) responded promptly to therapy. Another patient with a hypometabolism apparently secondary to a rare type of pituitary deficiency did not bleed after therapy. The authors state again that the percentage of cures is inversely proportional to the severity of the amenorrhea.—*E.C.H.*

REIDENBERG, L.

Low-dosage irradiation to the pituitary gland and ovaries in amenorrhea and dysfunctional uterine bleeding. *Am. J. Obst. and Gynec.* 45: 971. 1943.

Results of low-dosage irradiation to the pituitary gland and ovaries of 136 patients are reported. Of 51 patients with intercurrent amenorrhea, 36 had a return of cyclic bleeding and four conceived within three months after the cessation of therapy. All four patients with delayed menarche, ages from 18 to 22 years, failed to respond to therapy. Of 37 patients with infrequent bleeding averaging 5.7 cycles per year, 29 were restored to normal cyclic bleeding and 28 pregnancies occurred in 22 women. Of seven patients with scant uterine bleeding, six of whom also complained of sterility, four had a return of average amount of flow and one had three pregnancies. Of 27 patients with excessive or prolonged flow, 16 were restored to normal and five pregnancies occurred. Of 10 patients with totally acyclic rhythm, 5 had a return of cyclicity

In no instance was it thought that the preconceptional irradiation therapy had any deleterious effect upon the generative organs or upon the offspring of the first generation.—*E.C.H.*

WILLIAMS, W. W.

The basal metabolic rate, basal body temperatures, and the ovarian cycle. *Am. J. Obst. and Gynec.* 46: 662. 1943.

Correlational studies were made of basal metabolic rates, ranging from a minus 34 per cent to a plus ten per cent, and basal body temperature curves determined for from two to 12 months. Four out of five patients with basal metabolic rates ranging from minus 22 per cent to plus 10 per cent had normal temperature curves. The remaining one had high temperature levels due to pregnancy. Of 17 women with basal metabolic rates between minus 15 per cent and minus 4 per cent who received thyroid substance to tolerance, 16 had normal temperature curves. One individual of this group presented low temperatures at ovulation in two of eight cycles. Of 13 patients with basal metabolic rates between minus 15 per cent and minus 34 per cent only one had an abnormal temperature curve. According to these studies, there is no correlation of basal metabolic rates and basal body temperatures and one cannot alter basal body temperature curves by the administration of clinically tolerated doses of thyroid extract.—*E.C.H.*

WINSON, S. G.

The lactogenic effect of prolactin in the human being. *Am. J. Obst. and Gynec.* 46: 545. 1943.

The lactogenic effect of prolactin was evalu-

ated in three groups of puerperal women. The first group of 20 treated and 20 non-treated patients who did not nurse their infants because of prematurity were submitted to breast-pumping every 8 hours. The treated ones received 100 international units of prolactin twice daily from the first through the fifth post-partum day. The average amount of milk obtained for treated women was more than twice that from the non-treated ones. In the second group 100 patients were treated and 100 similar patients served as controls. All were required to nurse their babies during the 10 days of observation. With prolactin, three-fourths of the infants regained or exceeded their birth weight within the 10 day period whereas without prolactin only one-sixth succeeded in doing this. The last group consisted of 50 lactating women who had had insufficient milk on the fifth to seventh postpartum days. Similar therapy was given. Seventy-two per cent obtained a good response and 20 per cent no response. Reactions to prolactin may consist of pain at site of injection or engorgement of the breasts. The former occurred in 10% of the patients and the latter in 12% of 170 patients. —*E.C.H.*

GONADS

ALLEN, E.

A report on sequential abortion. *Am. J. Obst. and Gynec.* 46: 70. 1943.

The author presents brief case reports, including treatment of 28 patients who had one or more previous miscarriages or who had bleeding in the first trimester of pregnancy. Progesterone therapy was associated with 15 living babies in 19 patients and anhydro-hydroxy-progesterone, 2 living babies in 8 patients.—*E.C.H.*

BARROS, N. MORAES, PAULO DE GODOY, AND DOMINGOS DELASCIO

Struma of the ovary. *Obstetricia y Ginecologia, Latino-Americanas*, 2: 114, 1944.

The author had the opportunity to observe a case of thyroid tumor of the ovary. Some considerations are set forth with respect to this curious blastoma, and a clear explanation is given of what must be really understood to be "struma ovarii." A thorough analysis of the histogenesis, anatomo-pathology, symptomatology, diagnosis, prognosis and therapeutics of this strange tumor is made by the author who supports its teratomatous origin and accepts for its classification that of Wegelin.—*Author's summary.*

BEECHAM, C. T.

Androgen therapy in pelvic malignancy. *Am. J. Obst. and Gynec.* 46: 849. 1943.

Testosterone propionate, in maximum doses of 25 milligrams intramuscularly daily, was administered to six patients with advanced pelvic malignancy with metastasis. Pain was relieved rapidly and almost completely in most cases, and the patient's general condition temporarily improved. The authors believe that androgen therapy should be evaluated further in similar patients.—*E.C.H.*

BLOOM, O. H.

Dimethyl-ether stilbestrol in the menopause and for the suppression of lactation. *Am. J. Obst. and Gynec.* 47: 692. 1944.

The menopausal study comprised forty-nine women, from clinic and private practice who presented themselves complaining of vasomotor instability, etc. Dimethyletherstilbestrol, 30 milligrams, was administered intramuscular weekly until subsidence of all symptoms. No other therapy was given. The average total dose was 90 milligrams. The average number of days free of menopausal symptoms was 138.

For suppression of lactation, 15 milligrams of this drug and 15 milligrams of diethylstilbestrol were injected intramuscularly and repeated in 48 hours. A total of 269 patients were treated; 245 received two injections and 24 required a third injection on the day of discharge. Nursing apparently was not instituted. Among the 245 patients treated with two doses, there were 29 instances of engorgment, but no total failures. There were four total failures in the group who received the third injection. It was noted also that the first menstrual period was delayed in 30 per cent of these patients.—*E.C.H.*

BROWN, R. L.

Effect on spermatozoa of tissue fluids encountered in the female reproductive tract. *Am. J. Obst. and Gynec.* 46: 873. 1943.

Vaginal secretions were secured from patients with no leucorrhea or vaginitis. Endocervical fluid was removed from the incised cervix following hysterectomy. Mucosal scrapings were taken from the corpus and fallopian tubes. Follic-

ular fluid from unruptured nearly mature follicles, fluid expressed from the corpus luteum and fluid aspirated from multiple ovarian cysts were collected. Ascitic fluid was also procured. The material thus obtained was mixed with human semen, one-half hour after ejaculation, in the ratio of one to one; and these and a control were incubated at 37° Centigrade. Both were observed microscopically for immobility of spermatozoa.

The effects of these fluids as compared with the controls are as follows: average control time in minutes was 696; for vaginal secretion, 402; for cervical, 691; for uterine, 697; for tube, 666; average control time was 675; for follicular fluid, 800; for corpus luteum fluid, 755; for cyst fluid, 744; and average control time, 1,190; for peritoneal fluid, 1,150. Human spermatozoa are not affected deleteriously by any of the female genital tract tissue fluids except the vaginal secretion. —*E.C.H.*

CARY, W. H.

A method of obtaining endometrial smears for study of their cellular content. *Am. J. Obst. and Gynec.* 46: 422. 1943.

A technic is presented for aspirating endometrial and endocervical secretions for the study of their exfoliated epithelial content as a diagnostic aid in intrauterine carcinoma and other conditions of the uterus.—*E.C.H.*

CINBERG, B. L.

Oral hormonal treatment of functional amenorrhea. *Am. J. Obst. and Gynec.* 46: 823. 1943.

Eighteen patients, five with delayed menarche, 13 with intercurrent amenorrhea of unstated duration and one probable surgical castrate were treated. Therapy consisted of five daily intramuscular injections of 0.33 milligrams of estradiol benzoate or five to ten days of one to three milligrams of diethylstilbestrol orally and then five daily doses of 60 to 80 milligrams of oral pregneninolone. Several consecutive courses, at monthly intervals, were given to each patient. A total of 62 series of treatment given to the 18 patients resulted in 55 bleedings. No patient with delayed menarche bled regularly after treatment. Five of the 13 with intercurrent amenorrhea bled cyclically after cessation of therapy for four to 14 months. All biopsies of the endometrium prior to therapy showed an estrogenic response. During therapy, three out of nine biopsies showed a mixed endometrium; the remainder was estrogenic.—*E.C.H.*

CONNALLY, H. F.

The effect of stilbestrol on puerperal morbidity and lactation. *Am. J. Obst. and Gynec.* 46: 125. 1943.

Diethylstilbestrol, 5 milligrams in oil, was administered intramuscularly to 442 unselected patients from the day of delivery to the day of discharge. There were 467 control patients. The incidence of puerperal morbidity (a rise in temperature to 100.4° F. on any two days of the puerperium) was 5.6 per cent in the treated and 20.1 per cent in the untreated patients. Attempts were made to carry out breast feeding in all patients but only 45.3 per cent of the treated patients could nurse their infants whereas 74.4 per cent of the control group could do so.

Another series of 372 patients was treated for nine days with a morbidity of 1.1 and 5.2 per cent among the white and colored patients respectively. Only 16.6 per cent of this group could nurse their young. Three hundred and thirty-three patients were treated for three days, using same daily dose, with a 6.0 per cent morbidity among the white and 10.2 per cent among the colored patients. Successful nursing was carried out in 49.6 per cent.

The mechanism by which diethylstilbestrol exerts this effect on the reduction of incidence of puerperal morbidity is not clear. The authors do not recommend the use of diethylstilbestrol as a routine prophylaxis against puerperal morbidity. —*E.C.H.*

CORNER, G. W. JR.

The absorption of steroid hormones from the oral mucous membranes, with special reference to the sublingual administration of progesterone. *Am. J. Obst. and Gynec.* 47: 670. 1944.

Solutions of crystalline progesterone in propylene glycol containing 20 per cent absolute alcohol (such that the concentration of the hormone was 10 to 20 milligrams per cubic centimeter) were used. Thirty-five assays for progesterone were done on rabbits and assays were done on five monkeys. The data suggests that for rabbits the ratio of sublinguinal to subcutaneous is at least, four to one and for rhesus monkeys the ratio of sublingual to intramuscular doses is four or five to one.—*E.C.H.*

FILLER, W. AND N. DREZNER.

The results of surgical castration in women under forty. *Am. J. Obst. and Gynec.* 47: 122. 1944.

Bilateral oöphorectomy was done in 41 patients, all of whom were under 40 years and 29 under 35 years of age. No patient had had previous symptoms of a spontaneous menopause. Abrupt onset of menopausal symptoms occurred in 85 per cent of these patients. Barbiturate controlled 75 per cent and almost 25 per cent required no therapy. The duration of symptoms was six months or less in 50 per cent and less than one year in 75 per cent. The authors are of the opinion that the distressing after effects of surgical castration in relatively young women frequently are exaggerated.—*E.C.H.*

HAMAN, J. O.

Pain threshold in dysmenorrhea. *Am. J. Obst. and Gynec.* 47: 686. 1944.

In this study, the average pain threshold, as measured by a sensimeter, of 100 dysmenorrheic women was compared with 100 non-dysmenorrheics, 100 post-menopausal women and 100 male subjects. Of the dysmenorrheic group, 50 were of the functional type and 50 of the organic type. Of the post-menopausal group, 50 were physicologic and 50 surgical. The average pain threshold of the dysmenorrheic group was the lowest. The functional ones had a slightly higher sensitivity than the organic ones. The highest threshold was found in women past the menopause. Those who had a history of previous dysmenorrhea had a slightly lower level than those who did not. Excluding the dysmenorrheics, pain threshold is not very markedly affected by sex.—*E.C.H.*

HENRY, J. S. AND J. S. L. BROWNE

The contraction of the human uterus during the menstrual cycle. *Am. J. Obst. and Gynec.* 45: 927. 1943.

Patterns of uterine motility during the menstrual cycle were studied in 10 patients using the Knaus Technic which employed Graz balloons, cannula, manometer and kymograph. Pregnanediol studies were carried out in seven of these patients.

In seven patients with adjudged normal ovarian and uterine function, two patterns of spontaneous uterine contractions and two types of response to pituitrin were noted. The first, or follicular pattern, was characterized by rather small, rapid and fairly regular spontaneous contractions, with pituitrin producing moderate rises in tone and amplitude. The second, or luteal pattern, showed larger, slower and less regular contractions, with pituitrin producing tetanic responses more and more readily as termination of the phase was approached.

In a surgical castrate patient, previously primed with estrogens, progesterone produced both a spontaneous type of contraction and a response to pituitrin which is fairly characteristic of the luteal phase.

Two patients with anovulatory cycles showed uterine motility and response to pituitrin which was characteristic of the follicular phase.

The authors state that the weight of experimental evidence and of experience is against the claims made for the use of progesterone in treating dysmenorrhea and after pains.—*E.C.H.*

HIRST, J. C.

Favorable response of advanced endometriosis to testosterone propionate therapy. *Am. J. Obst. and Gynec.* 46: 97. 1943.

Two patients with proven advanced endometriosis, in which further operation was refused, were treated with testosterone propionate in dosages as large as 25 milligrams every third day in one patient and 150 milligrams in the other. Definite masculinization was observed in the latter patient. Both patients improved subjectively and the endometrial masses were believed to have decreased somewhat in size.—*E.C.H.*

HOWARD, J. W., L. T. JANSEN AND W. T. SALTER

Studies in cancer VIII. Stilbestrol and certain steroids in relation to tumor growth resistance. *Cancer Research* 4: 337. 1944.

Comparative studies were made on the effectiveness of estrone, diethylstilbestrol and progesterone in inhibiting the growth of implanted sarcoma 180 in mice. In certain strains estrone was effective in both males and females; in this strain stilbestrol was effective only in the females. Progesterone was inactive even in high dosage. In mice strains having a high incidence of spontaneous tumor, estrogens were totally ineffective.—*D.A.McG.*

JENSIK, R. J. AND F. H. FALLS

Mesonephroma of the ovary. *Am. J. Obst. and Gynec.* 46: 810. 1943.

A case report is presented of a mesonephroma of the ovary. About 27 reports have appeared in the literature. This tumor can occur at any age, produces no endocrine stigmata, is usually a

rapidly growing tumor that responds poorly to radiation therapy. This patient died less than one year after onset of symptoms.—*E.C.H.*

KAMINESTER, S.

Experience with the six hour rat test for pregnancy. *Am. J. Obst. and Gynec.* 47: 265. 1944.

This test was applied to 107 patients following the technic of Salmon and Geist. In 62 pregnant women, there were 61 positive tests. Friedman tests were run on 19 of these same patients and 18 were positive. Therefore, one patient, clinically pregnant, had a negative Friedman but a positive six hour test and one patient presented the opposite findings. Both tests were negative in one patient with a non-viable fetus. In 44 non-pregnant women and males, 43 were negative. The one positive was thought to be due to inexperience of interpreting the findings. Of this group, nine had also Friedman tests and all were negative. The author believes this method warrants further clinical trial.—*E.C.H.*

KROGER, W. S. AND S. C. FREED.

The psychomatic treatment of functional dysmenorrhea by hypnosis. *Am. J. Obst. and Gynec.* 46: 817. 1943.

Two psychomatic approaches were used. The first consisted of induction of hypnosis after rapport was established. This was characterized by a state of generalized hypersuggestibility which is about twice that of the normal waking state. Suggestions are made in this state that the next menses may be free of pain or without excessive discomfort and that the next menses will be normal in all respects. Posthypnotic suggestions last about a month, and when repeated, the desired effect may be permanent. All four patients were cured permanently using this method, only one treatment being necessary in two patients and from three to twelve for the others. The second method employed age regression with hypno-analysis. The patient is reverted to an age prior to the onset of dysmenorrhea and then slowly reoriented to the present chronological age. This allows for the extraction of many intimate facts that would ordinarily not be available to the physician with the patient in the awakened condition. Appropriate suggestions then are made for their removal. Five patients were treated by this approach and three were cured permanently; one was partially relieved. The one failure is ascribed to factors beyond control. The authors state that these forms of psychotherapy, which act by raising the pain threshold directly or through the eliciting of psychogenic factors indirectly causing the pain, provide a convenient and effective therapeutic procedure for curing permanently functional dysmenorrhea.—*E.C.H.*

LYON, R. A.

Management of the climacteric with ethinyl estradiol. *Am. J. Obst. and Gynec.* 47: 532. 1944.

Forty-five women with an average age of 64 years, individual ages ranging from 38 to 54 years, who complained of climacteric symptoms for an average of three years were studied for a control cycle and then during a number of estrogen treated cycles. Ethinyl estradiol. 50 micrograms, was given orally daily in series of 21 days, duration; usually a week free of therapy was allowed to follow. By this regime, these patients were effectively managed for 348 cycles of treatment. Transitory sequellae of mild degree occurred in 11 per cent of these patients some time during the estrogen therapy.—*E.C.H.*

MARTIN, P. I.

Detection of ovulation by the basal temperature curve with correlating endometrial studies. *Am. J. Obst. and Gynec.* 46: 53. 1943.

Basal temperature curves were studied during 181 cycles and, in 56 of these, premenstrual endometrial biopsies were done. Biphasic curves were found during 164 cycles and in all 47 of these cycles, in which biopsies were done, ovulation was diagnosed on the basis of secretory endometrium. During 17 cycles, monophasic curves were obtained and during each of 9 cycles, which were checked by biopsy, a proliferative type of endometrium was found, indicating that ovulation had not occurred.—*E.C.H.*

MCELROY, R. C., E. G. SNYDER AND J. H. CLARK

Clinical effects of the synthetic estrogen, hexestrol. *Am. J. Obst. and Gynec.* 46: 446. 1943.

Hexestrol, in daily doses of 2.0 to 6.0 milligrams, was given to 42 surgically castrate women whose ages varied from 18 to 54 years. Of 33 adequately treated women who had hot flushes, 20 obtained partial control and 13 complete alleviation. No vaginal smears are reported. A daily threshold dose of 6.0 milligrams was established which the authors believe may be given over

long periods without serious side reactions.—*E.C.H.*

MURPHY, D. P.

Prolongation of pregnancy and excessive fetal development following administration of corpus luteum extract in the treatment of threatened abortion. *Am. J. Obst. and Gynec.* 47: 697. 1944.

A case report of a 31-year-old woman who had had a previous spontaneous pregnancy. During this pregnancy, for four months while uterine cramps were present, she remained in bed and took daily orally 14 grains of corpus luteum extract. A total of some 1,500 grains was taken. Cesarian section was done after the patient failed to progress after 36 hours of labor, spontaneously originating 26 days after expected date of confinement. The fetus weighed 4,990 grams. The author wonders whether or not the large doses of corpus luteum extract given were instrumental in delaying onset of labor.—*E.C.H.*

MURPHY, J. A.

On the employment of octofollin for the relief of menopausal symptoms, for the suppression of lactation, and in gonorrheal vaginitis. *Am. J. Obst. and Gynec.* 46: 146. 1943.

Of 37 patients with spontaneous menopause, 24 reported complete relief of symptoms on daily oral dosages of one to twenty milligrams of octofollin. Of 16 patients with artificial menopause, 9 were completely relieved by dosages of two to ten milligrams daily.

Lactation was suppressed in 20 patients by the use of 15 milligrams daily and orally for four days. Apparently nursing was not carried out.

Fourteen girls ranging from eight months to five years in age were treated daily and orally with two milligrams of daily and vaginally with three milligrams for gonorrheal vaginitis. In each patient, smears were negative for the gonococci in one week and vaginal discharge and itching disappeared in three to four weeks.

Gastrointestinal symptoms due to administration of the drug were not frequently encountered.—*E.C.H.*

NEUSTAEDTER, T.

The value of mixed conjugated estrogens from pregnant mare's urine in the treatment of menopause. *Am. J. Obst. and Gynec.* 46: 530. 1943.

Fifty-three climacteric patients ranging in age from 32 to 55 years were treated with sodium estrone sulfate. Of these, 11 were surgical castrates and 42 had had spontaneous menopauses. All patients received 3.75 milligrams daily until all flushes were completely under control. The maintenance dose varied from 1.25 milligrams daily to 2.5 and 1.25 milligrams on alternate days. Treatment was continuous except during menstruation or when periods of bleeding occurred. All patients responded satisfactorily; only two did not tolerate the drug well. This therapy was thought to have caused bleeding in 5 patients. There was no correlation between clinical response and changes in vaginal smears.—*E.C.H.*

NEUSTAEDTER, T. AND L. L. MACKENZIE

The comparative value of endometrial biopsies and vaginal smears. *Am. J. Obst. and Gynec.* 47: 81. 1944.

This is a review of the practicality of these two methods for objectively determining ovarian function. The author states that the vaginal smear, in most instances, will yield correct information regarding estrogen elaboration during the proliferative phase of the menstrual cycle. However, if information regarding progestational activity or ovulation is sought, the endometrium serves as a reliable indicator.—*E.C.H.*

NOVAK, E.

The constitutional type of female precocious puberty with a report of 9 cases. *Am. J. Obst. and Gynec.* 47: 20. 1944.

A general review of the causes of female sexual precocity is given. Nine patients of the constitutional type are presented. Their menarcheal ages ranged from 15 months to seven and one-half years. All these patients continued to bleed more or less cyclically as long as they were followed. The author believes, on the basis of microscopic study of ovarian biopsies of several of these patients, that they may not only menstruate at early ages but may ovulate at abnormally early ages.—*E.C.H.*

PAPANICOLAOU, G. N. AND A. A. MARCHETTI

The use of endocervical and endometrial smears in the diagnosis of cancer and of other conditions of the uterus. *Am. J. Obst. and Gynec.* 46: 421. 1943.

A comparison is made between endocervical and endometrial smears and those obtained from

the vagina in the diagnosis of intrauterine cancer and other conditions. The authors believe the vaginal smear presents a distinct advantage because of its simplicity and the facility with which it can be applied as a general routine method of diagnosis.—*E.C.H.*

POTTER, J. C.

Chiari's syndrome. *Am. J. Obst. and Gynec.* 47: 276. 1944.

A brief case report is presented of a woman 32 years of age who did not menstruate following delivery and who continued to lactate. Some seven years later, she stopped lactating spontaneously and her menses returned. She became pregnant some two years later and following delivery has failed to bleed for 14 months and her breasts have remained dry. This is thought to be the first patient with Chiari's syndrome who has had a subsequent pregnancy and delivery.—*E.C.H.*

RAKOFF, A. E., K. E. PASCHKIS AND A. CANTAROW.

Conjugated estrogens in human pregnancy serum. *Am. J. Obst. and Gynec.* 46: 856. 1943.

A technic is given for the extraction, hydrolyzation and assay of blood serum for estrogens. In the eight specimens, from women who were examined in the last two months of gestation, the amount of combined hormone ranged from 23 to 50 per cent of the total estrogen content. The authors consider that the estrogens of pregnancy serum are intimately "bound" to the proteins fraction because: 1) vigorous and prolonged hydrolysis is required to free the combined hormone; 2) protein-free filtrates are practically free of any estrogenic content and almost all of the hormone can be recovered from the protein fraction; 3) the estrogens do not pass through a colloidal membrane; and 4) they are not freed by tryptic digestion.—*E.C.H.*

ROCK, J. AND A. T. HERTIG

Information regarding the time of human ovulation derived from a study of 3 unfertilized and 11 fertilized ova. *Am. J. Obst. and Gynec.* 47: 343. 1944.

In computing the probable time of follicle rupture from 3 unfertilized eggs, data were derived from endometrial histology, histology of the follicle or the recent corpus luteum, and cytology of the ovum itself. Data on 2 tubal eggs indicated that whereas the preovulatory phases varied from the ninth to tenth and eleventh to twelfth days, the corresponding computed postovulatory phases were identical: 15 to 14 days. Data on a ripe follicular egg indicated that ovulation was due between the eleventh and twelfth days and that the postovulatory phase would have been somewhat more than 14 to 13 days long.

Of 11 fertilized ova, eight or 72.7 per cent had an estimated postovulatory phase within 14 ± 2 days. Of the three exceptions variation from the outermost limits of the theoretical range was one day or less.

Data from three unfertilized ova and from 11 fertilized ova, indicate that ovulation takes place about 14 days before the first day of the next expected period.—*E.C.H.*

SANES, S. AND A. F. LIBER

Ectopic decidua in vermiform appendix. *Am. J. Obst. and Gynec.* 46: 719. 1943.

In the two cases reported, ectopic decidua was found in acutely inflamed appendices. In both instances a diagnosis of pregnancy was made by the pathologist without clinical information. —*E.C.H.*

SIDDALL, R. S.

Iodine vapor technique versus carbolfuchsin stain for vaginal smears. *Am. J. Obst. and Gynec.* 47: 260. 1944.

The technics of the two methods are described. The vaginal smears were obtained each day from 21 definitely post-menopausal women with varying degrees of estrogenic deficiency. Some of these were treated later with thyroid or various estrogenic substances. Comparisons indicate no constant or significant differences in grading or reaction for these duplicate smears. In view of its rapidity and simplicity, the author favors the iodine vapor technic.—*E.C.H.*